The Journal of HEREDITY

DUCKY—A NEW RECESSIVE MUTATION

(SEE PAGE 27)

Neurospora Phenocopies

Tomato Genetics Nomenclature

New Mutations in Rats and Mice

VOLUME 46 JANUARY-FEBRUARY, 1955 NUMBER 1

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The JOURNAL OF HEREDITY is published bi-monthly by the AMERICAN GENETIC ASSOCIATION at 32nd Street and Elm Avenue, Baltimore 11, Md., and 1507 M St., N. W., Washington 5, D. C. Address all communications regarding editorial matters to 1507 M St., N. W., Washington, D. C. Communications regarding the business management of THE JOURNAL OF HEREDITY may be addressed to the publication office, 32nd Street and Elm Avenue, Baltimore, Md., or to 1507 M St., N. W., Washington, D. C. Entered as second-class matter February 24, 1923, at the postoffice at Baltimore, Maryland. Acceptance for mailing at the special rate of postage provided for in section 1103, Act of October 3, 1917, authorized December 29, 1918.

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$7.00 a Year. $1.50 a copy. Prices of back numbers on application.

The

Journal of Heredity

Vol. XLVI JANUARY-FEBRUARY, 1955 No. 1

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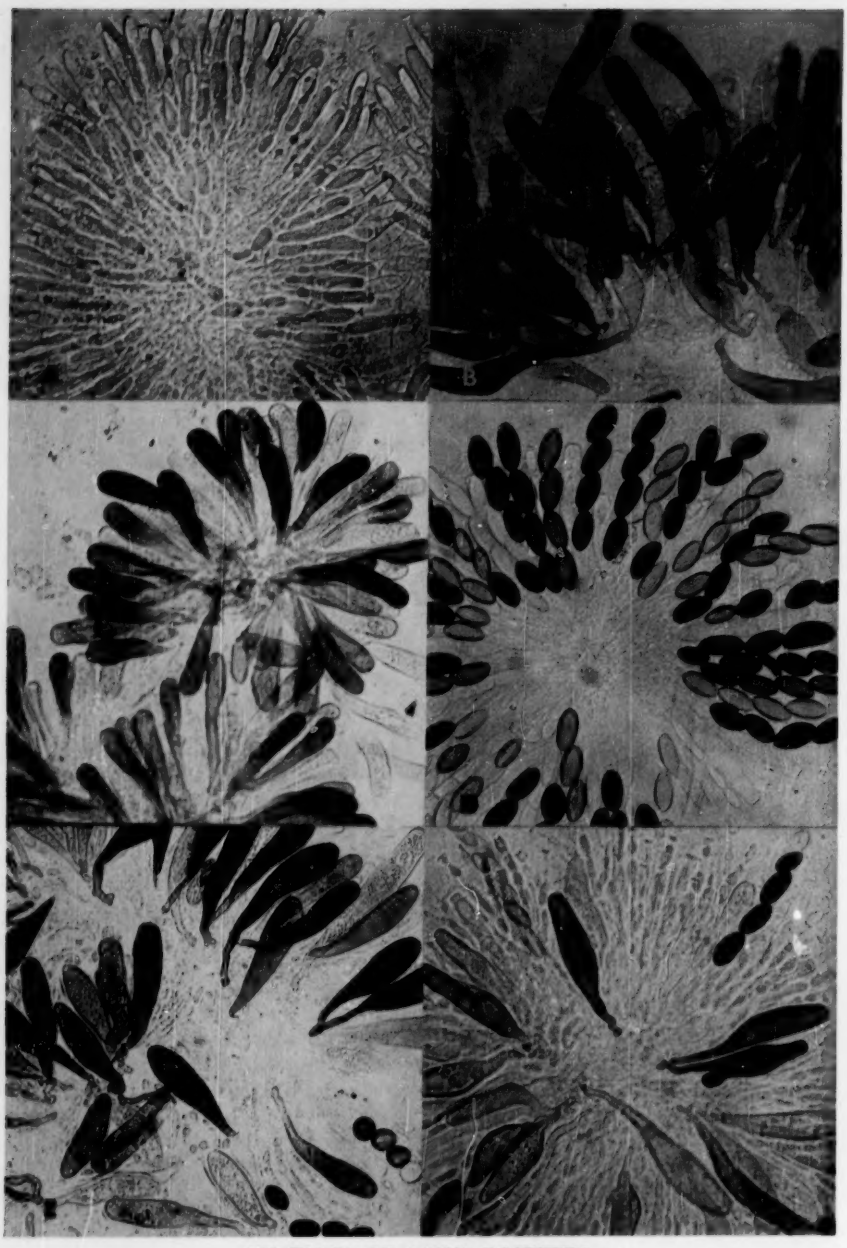

SIX TYPES OF ASCUS ROSETTES

Figure 1

The genotypes of the originally diploid asci are arranged in positions corresponding to the six ascus rosettes; magnifications vary somewhat. *A—Aaiidd; B—AaIidd; C—Aaiidd* (phenocopy of *B*); *D—AaiiDd; E—AaiiDd* (phenocopy of *F*); *F—AaIiDd.* These represent the six different types of ascus rosettes in *N. tetrasperma,* as adapted from Dodge and Seaver.[10] *Aa* = mating type genes; *Ii* = "Dominant lethal," *I* for indurated ascus abortion and its recessive wild type, *i*; *Dd* = dominant wild type, *D*, and recessive gene *d*, for delinquent ascus abortion. Rosettes *A* and *C* have the same genotype; *D* and *E* have identical genotypes; asci in rosette *C* are true phenocopies of asci in rosette *B*; aborted asci in rosette *E* are true phenocopies of aborted asci in *F*.

PHENOCOPIES IN NEUROSPORA

B. O. DODGE*
The New York Botanical Garden

THE term phenocopy implies a phenotype that is nonheritable but mimics or copies a phenotype that is heritable or genetic. The rumpless fowl of Dunn and Landauer[12] is a good example. Landauer[14] found that when insulin is injected into the yolk sac the chick may develop rumpless, resembling individuals of the breed rumpless. The difference here is that the induced rumplessness of the chick is nonheritable. Landauer has a clear discussion of the subject and a good list of literature.

While culturing races of *Neurospora tetrasperma* during August and September, 1926, it was noticed that some of the crushed perithecia showed several monstrosities among asci that were maturing spores normally. These curious bodies without spores were about the size and shape of full grown asci, perhaps a little larger. They differed from asci in having thick brown chitinous walls which were beautifully striated (Figure 2 *B* and *C*), thus resembling giant ascospores. Seeking to prove whether or not these ascus-like structures were genotypic and therefore heritable, sub-cultures were made and ascospores from the same perithecia were germinated and cultured. In every such test the perithecia obtained all developed perfectly normal asci with spores.

About a year later, perithecia from several different crosses showed many of these brown sporeless asci. One of them was illustrated by Dodge[2] in his account of the production of interspecific hybrids. The only mention he made of them was in the legend for his plate 3, Figures *F* and *G*. "Olive-brown indurated ascus showing anastomosing ridges similar to those usually found on the walls of ascospores; no spores develop in such asci. The cause for the formation of these aborted asci has not been determined. Compare *G*, a single-spored ascus."

In this discussion an ascus that does not delimit any spores is said to abort. It will be shown later that the indurated asci mentioned above are true *phenocopies* of an indurated type of ascus abortion that is heritable because such asci are heterozygous for a so-called "dominant lethal" *I*. However, before considering this heritable type, mention should be made of the unpredictable appearance of the phenocopies in *Neurospora* cultures at various times since 1926.

In order to better preserve evidence on the sporadic appearance of this nonheritable ascus abortion a number of slide mounts were made. For example, six of the earlier mounts, still in excellent condition, are very interesting because the labels indicate that these abnormal asci appeared so frequently, particularly in certain backcross hybrid asci. The labels are as follows: "S6 × 273, nonheritable 8-18-27 to 9-5-27"; "S6 × 242, nonheritable 8-8-27 to 9-5-27, Black asci"; "S6 × 238, 34 days old, abnormal, 9-21-27," (this slide shows about 100 indurated asci in a single perithecium); "256 × 233, backcross asci 9-19-27 at 15 days"; "262 × 238, abnormal asci F_2 haplonts, backcross, 9-22-27"; "S6 × 246 backcross, second generation." The race S6 in the above crosses was a normal tester race of *N. tetrasperma*, mating type *A*; the other haploid parent in each mating was a second generation backcross segregate of *N. sitophila*.

When S1 and S6 are mated and aborted asci suddenly appear as they did in two sets of cultures March 18 and March 25, 1929, the aborted asci are shorter and thicker than they are when

*The writer is indebted to Prof. W. J. Robbins, Director of the New York Botanical Garden, who extended the facilities of the Laboratory and other assistance for the continuation of our work on *Neurospora*. Prof. Francis J. Ryan of Columbia University read the manuscript critically. The writer highly appreciates his criticisms and valuable suggestions.

NEUROSPORA TETRASPERMA

Figure 2

A—Heritable indurated abortion of asci originally heterozygous, *AaIi*, abortion due to "dominant lethal" *I;* asci A_8, A_4 at right would later have become striated and indurated, the spotted or dappled appearance of the walls indicates early stages in the formation of striae. On Difco potato-dextrose agar such asci often deliquesce and disappear. *B* and *C*—Non-heritable phenocopies of asci such as those in *A;* note the brittleness of the ascus wall shown in *C* where two crossed asci collapse under pressure. *D*—Details of wall striations and characteristic ascus shaped like that of a tadpole. Note the numerous pale dots on the wall of ascus at the lower right. This shows a very early stage in the development of the striae. Magnifications vary somewhat.

S6 is backcrossed with a second generation *N. sitophila* segregate. Whenever new cases of indurated asci were seen tests were always made hoping to prove whether or not this induration of asci without spores was heritable as such.

Dodge[6] had proved that heterozygous asci, *AaDd* would delimit four spores

rather normally, but asci homozygous, *dd*, for this recessive lethal all abort and eventually deliquesce. Dodge and Seaver[10] while working with races C_4 and C_8 of *N. tetrasperma*, both of which carry recessive lethal *d*, observed some very striking examples of the nonheritable indurated type of ascus abortion. Because of some specific cultural disturbance in a basket of fifteen cultures, practically every ascus in the perithecia in seven cultures aborted and became indurated (Figure 1 *C*), while about 90 percent of the asci in the other eight cultures aborted and became indurated, but a few asci developed spores (Figure 1 *E*). Their records showed all asci in the seven cultures were originally homozygous, *Aadd*, while all asci in the eight cultures were heterozygous, *AaDd*. Theoretically, there should have been no abortion in these *AaDd* asci because factor *d* is recessive as noted above. Evidently the temporarily disturbing effect here was superimposed on effects of lethal *d*. The causal agent affecting indurated ascus abortion works independently in asci either *AaDd* or *Aadd*. The effects of bringing in the dominant lethal *I* for indurated ascus abortion will be shown later on. At that time the six ascus rosettes shown in Figure 1 will be compared and explained.

Germination of Indurated Aborted Asci

Dodge and Appel[7] reported other instances of nonheritable ascus abortion. Race dwarf 16 (*avD*) was crossed with mutant race C4 (*AVd*). The F_1 and F_2 haploid progeny were tested for mating type, *A/a*, yellow dwarf 16, *V/v*, and recessive lethal, *D/d*. The two testers C4 and C8 were used because all asci following from the test matings would thus carry the factor *d*, either as heterozygous, *Dd*, or homozygous, *dd*. Not infrequently, especially if the culture medium was a certain potato-dextrose agar, a perithecium would contain some of the nonheritable indurated type of asci. These authors showed clearly in their Figure 1, *C-E*, that indurated aborted asci originally homozygous, *AaVvdd*, for the recessive lethal *dd* could be germinated. All perithecia from their mycelial growths showed only the deliquescent type of abortion, no induration. This proved that an ascus with an indurated wall and no spores may still, for a time at least, contain living nuclei of opposite mating types *Ad*, *ad*.

During the winter months of 1951-52 many hundreds of test matings were made involving the pleiotropic gene *E* and the recessive *d* for deliquescent ascus abortion. The records show that practically all the asci *AaEedd* in the perithecia derived from eight entirely different matings aborted and became indurated. These asci were also true phenocopies of the heritable type due to the dominant lethal *I* described below.

Heritable Indurated Ascus Abortion

In point of time the phenocopy in *Neurospora* holds priority. That is, this type of ascus abortion was observed in 1926, then for eight years the writer took advantage of their occasional reappearance to try to find some definite gene combinations that would make it heritable. The writer is indebted to Dr. David Goddard for the culture, G5.3, of *N. tetrasperma* that served this purpose most beautifully. Uber and Goddard[17] had obtained the culture from an X-rayed ascospore. When received it was weakly bisexual.[5] The mycelium grew slowly and produced only a few conidia. Rarely a perithecium eventually developed a few ascospores. When grown with our tester S1, mating type *a*, a number of perithecia matured asci. On corn-meal agar about 75 percent of these asci aborted and became brown and striated. As in all previous cases sub-cultures were made and ascospores were cultured. Now every one of the perithecia in these new cultures produced many indurated asci without spores. Race G5.3 was maintained in sub-cultures for several years, but was eventually lost. It had served its purpose very well because it reacted as practically unisexual, mating type *A*. This very important "dominant lethal" *I* has also been preserved through many generations from ascospore to ascospore. Until recently it was thought that nuclei carrying this gene had to be accompanied in the same spore by a normal compensating nucleus. Heterocaryotic mycelia from such spores grow very well. It may be reported here that the writer has succeeded in obtaining pure cultures of unisexual races of both mating types *AI* and *aI* by germinating small unisexual spores and then growing the germlings on a new potato-steep dextrose medium.*

After culturing heterocaryotic races *AI-ai*,

*One characteristic of a dominant lethal gene is that it ordinarily prevents the haploid cell or gamete from functioning. As was expected great difficulty was encountered in obtaining germlings *AI* or *aI* that would live and function in matings. The data covering the details of this work, and the interesting facts relating to the many test cultures, are too extensive to be inserted here. They will be publishd at a later date.

and *Ai-al*, intermittently for 18 years it must be admitted that, like many "dominant" genes, this gene *I* is apt to show a percentage of dominance which varies anywhere from 100 percent to zero, or perhaps, even become recessive! Cultured on new-potato-steep-dextrose agar there is now no ascus abortion, but on Difco potato-dextrose agar the gene still proves to be 90+ percent dominant. On the latter medium many asci fail to become permanently indurated or even at all indurated. We have called these aborting asci "tadpole" because of their characteristic shape, (see Figures 2 *D*, and 1 *B*, *C*, *E* and *F*).

In addition to further examples of the nonheritable indurated asci mentioned previously, Dodge and Seaver[10] described in some detail how they obtained the heritable type by way of matings that would provide asci that were *AaIiDd*, while other matings gave asci that were *AaIidd*. By comparing in two ways the six different ascus rosettes in Figure 1, that is, considering both their phenotypes and genotypes, one sees that the asci in *C*, originally *Aaiidd*, are all phenocopies of asci in *B*, which were *AaIidd*. Furthermore, all the indurated sporeless asci in rosette *E*, *AaiiDd*, are true phenocopies of the indurated asci in rosette *F*, *AaIiDd*. Furthermore, the 4-spored asci in *E* are phenocopies of the 4-spored asci in *F*. This is true because if one cultures from an ascospore in *E* the progeny rosettes will be like *D* and not like *E*. Culturing from an ascospore from *F* the progeny rosettes will be like *F* itself. In other words rosette *E* as a whole is a phenocopy of rosette *F* as a whole.

Asci in rosette *A* were all originally *Aaiidd*. The eight haploid nuclei in each ascus degenerate.[6] Asci in rosette *C* had the same genotype *Aaiidd* as those in *A*. One curious and important effect of the nonheritable indurating agent is that it more or less prevents the degeneration of the eight nuclei in asci homozygous *dd* for the recessive lethal *d*, then stimulates those eight nuclei to divide again. As noted previously, Dodge and Appel[7] actually germinated some of those indurated asci like those in rosette *C* and found that asci in the new generation all aborted like those in rosette *A*, no induration. Asci in *C* all *aborted*, — they were sporeless. Yet some of them must have lived to *"germinate"* and function in reproduction. They must have been heterocaryotic at the time, having living nuclei of two kinds, *Ad* and *ad*. Asci like those in rosette *A*, Figure 1, originally being *Aaiidd*, must eventually abort whether or not their eight nuclei can be prevented from degenerating. If the asci are like those in rosette *B*, *AaIidd*, they will, so far as yet known, not only abort, but their walls will become more or less indurated.

Discussion and Conclusions

Regardless of whether or not indurated abortion of asci is nonheritable, or is heritable due to the dominant gene *I*, failure to form spores might be a matter of timing for the fourth nuclear division which normally occurs *after* spore formation. In the Ascobolaceae one finds that there may be as many as seven successive simultaneous equational nuclear divisions. The number of such divisions is *specific*. There are species with 8, 16, 32, 64, 128, 256, 512 and 1024 spores respectively. But, species of *Neurospora* are all timed to delimit their spores only after meiosis and one equational division, giving, in all, eight nuclei. Anything like gene *I* stimulating the fourth division to occur *before* spore formation must, it seems, result in ascus abortion. One can upset this timing due to gene *I* by culturing on a new-potato-steep-dextrose or other favorable medium, where there will be little or no abortion. The numerous ascospores matured will be mostly heterocaryotic, *AI-ai*, or *Ai-al*.

Wilcox[18] diagrammed (her Figure 2) the various positions of the eight spores in asci of *N. sitophila*, arranged according to sex factors. It so happened that in the few asci she analyzed the spores alternated two and two (or two-four-two) for mating type factors. She pointed out that Dodge had also assumed from his previous studies that these factors segregated in the second nuclear division in asci. The point here is that neither of them had analyzed enough asci. Dodge[3,4] later found that these factors also segregated in the first meiotic division. Lindegren[15] proved that the two-two and two-four-two arrangements compared with the four-four distribution actually do place the points of segregation so that the percentages can be accepted as measures of crossing-over and therefore chromosome map-distances in *N. sitophila* and *N. crassa*. These spore arrangements are, therefore, phenotypic expressions of cytogenetic facts. Does this statement hold for the spore arrangement picture in the eight-spored mutant race of *N. tetrasperma*? Dodge, Singleton and Rolnick[11] analyzed the eight spores from many asci that were originally heterozygous *AaEe*. Considering only *A/a* and *E/e* factors they found a great preponderance of the two and two (two-four-two) arrangement and practically no clear-cut example of the four-four type. However, since asci with four genotypically different spores, *AE*, *Ae*, *aE*, *ae*, were also very rare the authors pointed out that: "Phenotypic second division segregation here is not at all synonymous with genotypic second division segregation." Their explanation was that mating type *A/a* and dominant *E/e* gene pairs are so close to their respective centromeres that crossing-over would be rather rare. So far as proved one could only say that the spore arrangement pictured here is an interesting genetic parallelism the expression of which is a response to a certain cultural environment. This spore arrangement, being to the eye similar to the pictures afforded by *N. sitophila*

and *N. crassa*, may turn out to be a good measure of crossing-over percentages if used in reverse!

Zickler[19] found that the introduction of chloral hydrate into his cultures of *Sordaria macrospora* and *Neurospora crassa* tended to induce the formation of giant ascospores. He says that some asci have only a single giant spore which completely fills the ascus, top to bottom. The structures which he figures as such are not giant spores. They are aborted indurated asci. By crossing the writer's new race "Ind. 52," *al*, of *N. tetrasperma*, with a normal *N. crassa* one could obtain, especially by backcrossing, asci heterozygous, *AaIi*, some of which would abort and become dark colored and indurated. Zickler's black sporeless asci would be true phenocopies of them. His chloral hydrate effects would certainly be comparable to Landauer's insulin induced phenocopies of rumpless fowl. One might go outside of *Neurospora* to point out a false phenocopy in the rust fungi.

In the rust *Gymnoconia interstitialis* the two nuclei in each cell of the young teliospore fuse to give the only diploid cells in the life cycle. Reduction occurs and a 4-celled promycelium is formed of which each cell gives rise to a basidiospore. In the short-cycle form "*Kunkelia nitens*" of this species there is no nuclear fusion.[8] The entire life cycle is haploid. As the binucleate aeciospore germinates the two nuclei migrate into the young promycelium and divide again. Then a four-celled promycelium is formed which phenotypically mimics the promycelium of the long-cycle ancestral form. This is at most merely an interesting case of mimicry well known to mycologists. If Atkinson[1] had accomplished what he sought to prove experimentally he would have given us an example of a true phenocopy in this most interesting of all the rust fungi.

The order of the appearance of the mutant and the phenocopy is definitely stated by Sinnott, Dunn and Dobzhansky.[16] "In cases in which the mutant change has been observed first and then an imitation or mimic is found to follow an environmental change, the mimic is sometimes called a phenocopy." This could not rule out the use of the term in cases where one found the nonheritable phenotype first and later the gene mutant. That could even have been the original order of procedure and progress in evolution.

Those 8-spored asci of *N. tetrasperma* mentioned previously are phenotypically excellent mimics of the normal asci of *N. sitophila*. They are, however, themselves heritable due to the gene *E* in heterozygous asci and are therefore the ones to be copied nonheritably. Whether or not this gives any good hint on how the 4-spored species of *Neurospora* were originally derived from the 8-spored remains an interesting question. Goldschmidt[15] believes that while the phenocopy tells us nothing about the origin of the hereditary type, "it does give highly important information regarding the potentialities of the organism." So it may be that both the true phenocopies and the phenocopy-like parallelisms in the fungi will throw some light on the evolution within this important group if properly studied and interpreted.

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APOMIXIS IN BLACK PEPPER AND JOJOBA?

THE black pepper, *Piper nigrum* L., is perfect-flowered, dioecious, or a little of both. At least since 1906,[1] authors of articles on pepper have either stated directly or implied that good fruit set depends largely on perfect-flowered plants. My recent observations of cultivated pepper varieties on the Malabar Coast of India throw a confusing light on this precept.

In the variety "kalluvalli" the flowers are perfect and it is known as a good setter. In the variety "balamcotta" some of the spikes on a single vine bear some perfect flowers, while other spikes are quite without stamens. The fruit setting was observed to be ragged, many of the ovules remaining undeveloped. In "uthirancotta," a variety reported as dioecious, I observed only pistillate plants. The set of fruit was quite poor, the inflorescence rhachis being interrupted by sporadic fruits and without the persisting ovules of "balamcotta." So far, the observations corroborate the assumption: perfect flowers, good yielder; fewer stamens, less yield; no stamens, very light yield.

However, in Travancore I encountered the variety known as "kotanadan" or "kudaravalli," Gentry collection Nos. 13511, 13565. The spikes were not only densely and completely filled with well developed fruits, but the bases of the spikes commonly bore two or three branches, making a compound inflorescence. But I could not locate a single stamen. *The plants were unisexual.* Not only does this repudiate in some way the concept of yield correlated with stamens, but suggests some unperceived factor operating to promote fruit development quite independent of pollination. According to Menon,[2] pollination is accidental, and thought to be effected by rain splash and carriage. There are no known insect pollinators. The pollen grains are reported to be small, so there may be wind carriage, although it was not mentioned by Menon. However, no staminate flowers were noted proximal to the "kotanadan" planting observed.

Jojoba, *Simmondsia chinensis* (Link) Schneider, of the Sonoran Desert of northwest Mexico, is well-known as a dioecious plant. In numerous articles,[3] it has been assumed that pollination, or the presence of staminate plants, is necessary for fruit set and development. As with pepper there are no known pollinating agents, such as insects or birds. The pollen in Jojoba is coarse and heavy, not of a wind-borne type. However, I have observed in several situations in various years solitary or isolated pistillate plants freely fruiting, with the nearest staminate plants as much as two or three miles distant.

Here are two species of dicotyledonous plants, quite unrelated as to family, in which unisexual flowers appear to develop normal fruits quite irrespective of pollination. So far as known, the viability of the seeds of such individuals has not been tested, but in appearance they are the same as those of known normal viability. The embryology of both jojoba and pepper needs study.

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THREE NEW MUTATIONS OF THE RAT

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WE report herewith on three new mutations, two of which originated in the Small Animal Laboratory of the Department of Genetics in Berkeley, the third (an important one) at the Illinois Institute of Technology in Chicago.

Shaker

1. Shaker (*sr*) is a mutation first observed by Drs. M. Lerner and E. R. Dempster in 1947, in an inbred strain of albino rats kindly supplied by Dr. Helen Dean King of the Wistar Institute.

Mutant individuals can be identified as early as 18 days after birth by their peculiar shaking locomotion, more like crawling than walking. They are of smaller size than their normal litter mates, and try to hide behind them, being very nervous and excitable, so that they may run about the cage in a frenzied way, if disturbed. They grow more slowly than their normal litter mates and rarely attain sexual maturity. An occasional male may breed successfully for a time, but the majority in both sexes are sterile and die before attaining the age of four months. The mutation is thus sublethal.

Its inheritance is that of a simple autosomal recessive. Tests for linkage between the gene for shaker (*sr*) and other genes have been made with the results shown in Table I, all being apparently negative.

The most doubtful case is the backcross involving genes *d* and *sr*. The reader may wonder why this was discontinued at so early a stage when the backcross population consisted of only 39 tested individuals, of which 12 were crossovers and 27 non-crossovers, an indicated crossover percentage of 30.7 ± 5.4.

A closer examination of the figures shows that there is no significant difference between the frequencies of the double recessive and the two single recessive classes, all of which are expected to be equal. The unbalance between crossovers and non-crossovers is due solely to the low frequency[1] of the double dominant class, which should show a maximum of vigor and viability. Hence the conclusion was reached that its low frequency was due to random sampling rather than to linkage. This particular case merits further investigation which is now in progress.

Cowlick

2. Cowlick (*cw*) is a recessive mutation first observed in 1947 by Dr. H. P. Donald of Edinburgh, when visiting the colony. The character was then present in a single female individual of a moderately inbred stock of pink-eyed hooded rats homozygous for genes *A*, *B*, *p* and *h*. The original female was bred to a brother, and her brothers and sisters to each other, so that a true breeding race of cowlick individuals was soon established and several linkage crosses were started.

The character consists of a hair whorl on the back (Figure 3 *C* and *D*). Normally the hair on the dorsal surface slopes backward from nose to tail. In a cowlick individual a reversal of the hair slope is found at a point lying near the middle of the back or between that point and a point on the back of the head just behind the ears. From the point of reversal the hair slopes outward in all directions for a certain distance. The character is most conspicuous when the center of reversal is well posterior, as seen in Figure 3 *C* and *D*. When the center is far forward on the neck, the character is observed only as a forward directed tuft of hair between the ears, which may escape observation altogether.

An extensive program of tests for

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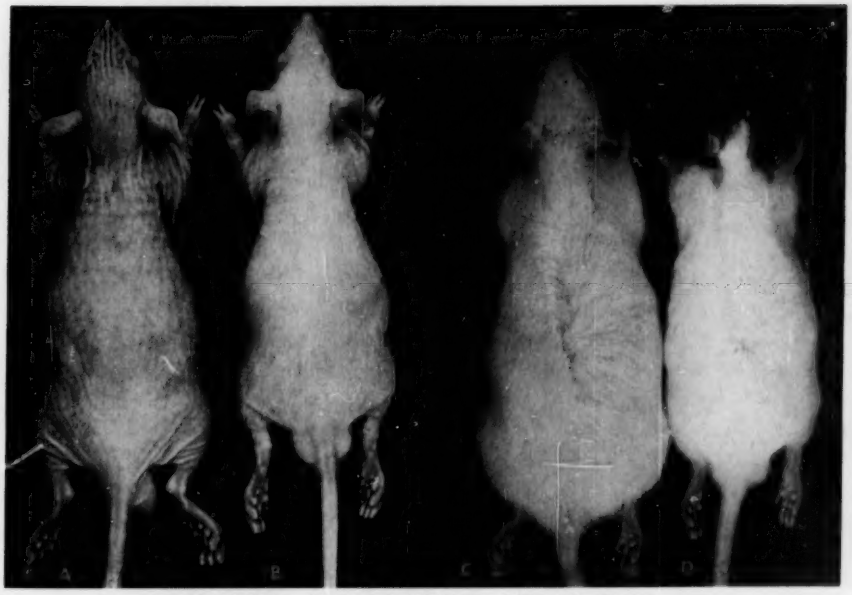
BLACK NAKED AND HAIRLESS RATS

Figure 3

The gray rat at the left, about four weeks old, shows loss of juvenile coat in progress. The black naked rat at the right at about four weeks of age, shows the fuzzy coat beginning to develop. In the center is a black naked rat about two months of age, the fuzzy coat is well developed but is beginning to be lost on the head.



HAIRLESS, NAKED HAIRLESS AND COWLICK

Figure 4

A and *B*—Litter mates about ten weeks old: *A*—Hairless—note the skin is beginning to wrinkle. Compare with Figure 5. *B*—Doubly homozygous naked hairless. The coat is fuzzy but the skin is smooth. *C* and *D* shows two cowlick rats: *C*—Albino; *D*—Black hooded.

linkage of cowlick with other genes (Table II) was carried out simultaneously with the linkage tests of shaker, but neither of these genes was tested for linkage with the other until nearly all other possibilities had been canvassed. Very much to our surprise cowlick and shaker appeared to be linked with each other on the evidence of an F_2 population of 244 individuals in which the recombination double recessive class was strongly deficient, as follows:

	sr cw	*sr*	*cw*	*o*
Observed	7	48	41	148
Expected	15.2	45.7	45.7	137.2

But further investigation, based on an outcross of an F_1 male to normal females, has shown this conclusion to be incorrect. The outcross was made to insure the production of a population of uniformly high viability, and the outcrossed young were then backcrossed to an F_1 animal to ascertain for each individual tested whether it carried gene *cw*, or gene *sr* or both or neither, the first two classes being non-crossovers, the last two crossovers. The totals for the 94 tested individuals was 46 crossovers: 48 non-crossovers, indicating clearly that genes *cw* and *sr* are independently inherited.

Naked

3. Naked (*n*). This new hairless mutation of the rat we shall call naked to distinguish it from the earlier described hairless mutation, although it might perhaps more appropriately be called fuzzy. It is a simple recessive character (gene *n*) and is linked with hairless (gene *hr*).

It made its appearance in April 1950 among the young of a pair of rats, the sole survivors of a crossbred stock employed by Dr. Shurrager in an experimental study of the relationship between body temperature and susceptibility to audiogenic seizures.

ADULT NAKED RAT

Figure 5

Dorsal and lateral views of an adult naked rat showing cyclical development and shedding of the fuzzy fur.

Dr. Shurrager established the fact that the mutation was inherited as a simple recessive character, and that naked rats were inferior to their normal litter mates in size and survival value. She then consulted the other authors as to its distinctness from hairless and we exchanged stock for a cooperative and comparative study of the two mutations with special reference to their linkage relations.

We independently discovered that naked individuals, unlike hairless, can be identified at birth by their bent vibrissae and this has been a great convenience in the linkage studies made in Berkeley.

Naked young, unlike hairless, develop only a scanty first coat and this is lost at about three weeks of age, when the skin acquires a yellowish crusty look and feels rough and dry to the touch. As the animals grow older the skin loses its crusty texture and becomes soft, pink (in albinos), and apparently healthy. Fuzzy hair appears at about the beginning of the fourth week and thereafter drops off and redevelops in cycles. In these cycles the new growth is first noticeable and is heaviest on the head. It is also heavier on the legs, underbody, and base of tail, although there is considerable variation in the amount and distribution of the hair in different individuals. With each successive loss of hair, less appears to be regained and after the sixth month some

ADULT HAIRLESS RAT
Figure 6

Adult hairless rat, showing the thick warty and wrinkled skin which is almost devoid of hair except for the vibrissae.

animals remain almost completely bare.

The hair differs from that of the normal rat in both length and texture. The coat of the naked rat is short and fuzzy. Under the microscope the individual hairs appear irregular in diameter and have a bent shape.

Hairless rats, on the contrary, develop a normal appearing first coat, which is lost at the age of about four weeks and is never fully replaced. The skin thereafter becomes thickened and wrinkled, increasingly so with age.

Naked is epistatic to hairless in rats six weeks of age or older. The double recessive genotype has at birth curly vibrissae, as do all *nn* individuals, but its skin remains smooth, not becoming thick and wrinkled as in ordinary hairless rats.

In both naked and hairless rats the vibrissae persist throughout life.

Both naked and hairless females are poor mothers because of a deficient milk supply, so that it has been found desirable to employ heterozygotes as foster mothers in breeding experiments.

A cross between naked and hairless stocks obtained by this procedure, resulted in normal appearing young carrying genes *n* and *hr* in the repulsion relationship.

An outcross was next made of F_1 with a normal stock carrying neither *n* nor *hr*, and the resulting young were backcrossed to F_1 to ascertain which of the two genes they had inherited, or whether they had inherited both or neither, the last two being crossovers. Forty-nine individuals of the outcrossed population were thus tested and they fall into the

TABLE I. Tests for linkage between shaker (*sr*) and other genes. The extreme classes are crossovers, the intermediate classes are non-crossovers.

Gene	*sr* x	x	*sr*	o	Expected ratio	Estimated Recombination Frequency*	P*
I *c*	25	91	26	82	1:3:1:3	.467	.64
I *p*	18	48	55	142	1:3:3:9	.496	.93
II *Cu*	43	101	28	93	1:3:1:3	.565	.22
III *hr*	9	9	36	92	1:3:3:9	.622	.05
IV *k*	17	19	20	16	1:1:1:1	.458	.48
V *A*	18	54	18	47	1:3:1:3	.474	.63
h	9	39	6	29	1:3:1:3	.520	.83
h	18	57	64	161	1:3:3:9	.468	.47
f	12	16	19	19	1:1:1:1	.470	.62
Ca	9	31	14	45	1:3:1:3	.487	.88
d	11	15	12	1	1:1:1:1	.310	.02
cw	7	48	41	148	1:3:3:9	.413	.07
cw	10	19	14	18	1:1:1:1	.459	.71
cw	13	27	21	33	1:1:1:1	.489	.90
Cu_2	9	35	23	42	1:1:1:1	.468	.70

TABLE II. Tests for linkage between cowlick (*cw*) and other genes, in repulsion crosses. The extreme classes are crossovers, the intermediate classes are non-crossovers.

Gene	*cw* x	x	*cw*	o	Expected ratio	Recombination*	P*
I *c*	37	29	29	34	1:1:1:1	.550	.25
I *p*	23	19	21	30	1:1:1:1	.570	.18
II *Cu*	19	30	29	50	1:1:1:1	.519	.58
II *Sh*	35	49	36	49	1:1:1:1	.497	.94
III *wo*	7	22	23	85	1:3:3:9	.523	.72
IV *k*	6	35	52	164	1:3:3:9	.414	.25
V *A*	45	47	29	26	1:1:1:1	.483	.51
Ca	24	58	10	25	3:9:1:3	.505	.94
Cu_2	29	23	25	23	1:1:1:1	.520	.69
d	6	27	22	71	1:3:3:9	.451	.47
h	75	56	72	81	1:1:1:1	.549	.27
f	18	28	22	87	1:1:3:3	.453	.44
sr	13	27	31	33	1:1:1:1	.489	.84

*Except for double backcross data, the product method formulae or tables (Immer[illegible]) are used. P is probability of obtaining the observed or greater deviation of expected recombination frequency from the 50 percent expected on the hypothesis of independent assortment.

following classes of carriers:

nhr	*n*	*hr*	*o*
8	16	16	9

Crossovers number 8 + 9 = 17, non-crossovers 16 + 16 = 32. The indicated crossover percentage is 34.7 + 4.8.

It was known from an earlier investigation[3] that hairless (*hr*) is linked with wobbly (*wo*) with a crossover percentage of 40.3 ± 3.1. This is greater than the indicated crossover percentage between *hr* and *n*, which would show that *n* lies closer to *hr* than does *wo* and that its probable position is between them in chromosome III.

To ascertain its position more accurately a repulsion cross was made between *n* and *wo* and F_1 individuals were outcrossed to a stock which carried neither gene, as had been done previously in the case of genes *n* and *hr*.

The outcrossed population was now backcrossed to F_1 to ascertain the proportion of crossover gametes which the F_1 parent produced, resulting as follows:

nwo	*n*	*wo*	*o*
20	18	24	25

Crossovers number 20 + 25 = 45; non-crossover 18 + 24 = 42. Indicated crossover percentage was 51.7 ± 3.6, a non-significant deviation from equality. In other words genes *n* and *wo* behave in transmission with apparent independence of each other, although both have been shown to be linked with the intermediate gene *hr*.

The linkage map of chromosome III would then become quite extended with tentative values as follows:

wo	*hr*	*n*
0	40.3	75.0

These estimates of map distance would undoubtedly be increased were intermediate genes available for detecting double crossovers in each of the two regions. In fact by applying Kosambi's[5] empirical approximation to these crossover data, we obtain map distances of 56 and 48 respectively for the two regions, or a total distance of 104 map units between *wo* and *n*. This same formula would estimate a crossover percentage of 48 for a map distance of 104 map units, which agrees fairly well with the observed percentage of 51.7.

A cross involving genes *wo* and *n*, made without knowledge of the intervening locus for gene *hr*, would have led to the erroneous conclusion that *wo* and *n* lie in different chromosomes.

Such a result would be no surprise to one familiar with the work of Bridges and Morgan[1] on the observed crossover values for a number of known second chromosome genes in *Drosophila* ranging between 46 and 55 percent. The same authors[2] gave curves relating map distance to crossover percentages for the third chromosome, showing that close to 50 percent recombination values are to be expected when map distances are as great as 100 units.

An interesting illustration of this same principle is found in the recent work of Kramer *et al*[6] on studies of linkage in barley. They find cytogenetic evidence that two linkage groups previously recognized and supposed to be independent actually lie at opposite ends of a single chromosome.

Summary

Three new mutations of the rat are described. All are inherited as simple autosomal recessive characters.

Cowlick is seen as a whorl of hair on the back, where normal rats have hair uniformly sloping backward.

Shaker is a manifestation of a nervous defect resulting in abnormal locomotion, timidity, and commonly in sterility and early death.

Naked is a new form of hairlessness.

Linkage studies have shown independent inheritance of all three mutants, with the exception of naked. Naked shows linkage with hairless, with a crossover percentage of 34.7 ± 4.8. It was previously known that hairless is linked with wobbly in chromosome III, with a crossover percentage of 40.3 ± 3.1. Yet naked shows no linkage with wobbly—crossover percentage 51.7 ± 3.6.

The explanation of this fact is assumed to be the long map distance between the genes for naked and wobbly both of which show linkage with the gene for hairless which lies at an intermediate locus.

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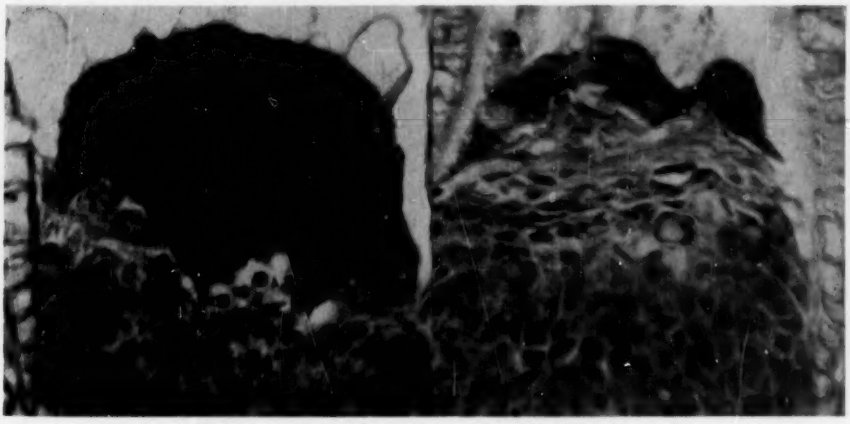
SEED DEVELOPMENT AND FAILURE IN HORSERADISH

G. W. STOKES*



CHALAZAL ENDOSPERM AND ADJACENT NUCELLUS

Figure 7

A—Normal interaction between the chalazal endosperm and the adjacent nucellus. *B*—Abnormal interaction between the chalazal endosperm and the adjacent nucellus.

HORSERADISH (*Amoracia rusticana* Gaertn.) is propagated by root cuttings, and although it flowers, seeds are rarely formed. There are two varieties of horseradish: 1, Bohemian, a clone found growing in fence rows and in some home gardens; and, 2, Common, a clone that is grown commercially. Weber[6] selected many clonal variations of both varieties from widely separated areas in Wisconsin. He used Common as the pistillate parent, since it fails to produce functional pollen. Bohemian produces some functional pollen and was used as the staminate parent. He harvested 12 viable seeds following approximately 10,000 crosses. One of the seedlings, plant C, proved valuable both as a male and female parent. The following year, plant C was used as the female parent in backcrosses with the Bohemian variety.

Since horseradish is easily propagated by root cuttings, each seedling was clonalized. No effort was made to use a single plant of a clone for work in the present study.

The present study was undertaken to determine the normal course of seed development in horseradish and the nature of seed failure when such occurs. The plant poses two major difficulties in that it is self-incompatible and the best cross available sets but few seeds. Normal seed development and seed failure in horseradish have been separated by

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observation alone, since a control was not possible for comparison. Both normally developing seed and those in the process of failure may be present in the same fruit.

Materials and Methods

Plants of clone C were used as the female parents, and plants of clone P-7 (from a backcross progeny C × Bohemian) were used as the male parents. When the plants were at the peak of their flowering cycle, the spikes were marked and the open flowers were pollinated. Collections following pollination were made at stated intervals up to 72 hours and at daily intervals thereafter through 21 days, at which time the seeds were near maturity.

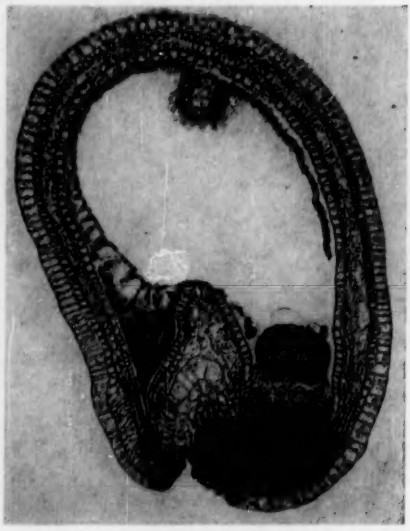
Ovaries and young fruits were collected and fixed overnight in a modified Carnoy's solution (3 parts 70 percent ethyl alcohol : 1 part glacial acetic acid) and then transferred to 70 percent ethyl alcohol for storage. The ovules and developing seeds were dissected from 3-day and older fruits prior to further processing. All materials were dehydrated in ethyl alcohol, cleared in cedarwood oil, and embedded in 50-52° C. paraffin. The seeds were sectioned longitudinally, mounted serially on slides, and stained with Delafield's Haematoxylin or safranin.

Pollen counts were made by smearing fresh, mature anthers in a few drops of aceto-carmine stain. Functional pollen grains become stained, whereas nonfunctional pollen grains do not. Percentages were calculated on counts of 300 pollen grains.

Observations

Fertility of the parents

The parents of the material used in the present study produced about 60 percent of what appeared to be functional pollen. This would suggest irregularities during meiosis. Although disturbances during microsporogenesis were observed, they followed no regular pattern. Abnormal female gametophytes were also observed which could account for the lack of seed formation in some instances. The number of such female gametophytes was low and no attempt was made to determine their frequency.



A GERMLESS SEED

Figure 8

Seed failure due to embryo abortion occurs less frequently than that due to endosperm failure. Abortion of the embryo early in ontogeny leads to germless seeds.

Seed development following the cross C × P-7

Fertilization takes place between 18 and 30 hours following pollination (Figure 9 *A* and *B*). The polar nuclei and a male gamete fuse to form the primary endosperm nucleus. The egg and the other male gametes fuse to form the zygote.

Mitotic division of the primary endosperm nucleus precedes that of the zygote. The endosperm is free-nucleate during the early stages of its development. The nuclei divide in mitotic waves that proceed from the micropylar toward the chalazal end of the endosperm. This pattern of mitosis gives rise to geometric multiples of nuclei at the close of each mitotic wave. The free-nuclei are for the most part peripheral but become congested in the micropylar and chalazal portions of the endosperm sac. The endosperm remains free-nucleate until about the time of cotyledonary initiation in the developing embryo. The endosperm first becomes cellular at its micropylar end. Cell wall formation follows as the mitotic wave proceeds toward the chalazal pocket (Figure 10 *A* and *B*).

A portion of the endosperm in the chalazal region immediately adjacent to the nucellus remains free-nucleate (Figure 10 *C*) and persists as a separate structure throughout the

later course of seed development. Haustorial outgrowths are formed from the chalazal free-nucleate portion of the endosperm. These project into and gradually digest the nucellar tissue.

The high frequency of prophase nuclei in the nucellus during the early stages of seed development suggests a build-up of that tissue. There is, however, a gradual reduction in the amount of nucellar tissue as the endosperm develops (compare Figure 10 *C* and *D*). Light-staining globules move from the chalazal portion of the endosperm into the central vacuole.

Division of the zygote (Figure 11 *A*) occurs between 36 and 48 hours following pollination and is associated with a 32 to 64 free-nucleate endosperm. Three and four-celled proembryos are accompanied by a 128 to 256 free-nucleate endosperm at 72 hours. The divisions within the proembryo are transverse forming a linear row of four cells.

The apical cell of the four-celled proembryo serves as the embryo mother cell, and the suspensor develops from the three remaining cells. The suspensor cells continue to divide transversely, forming a row of 13 to 15 cells by six days after pollination.

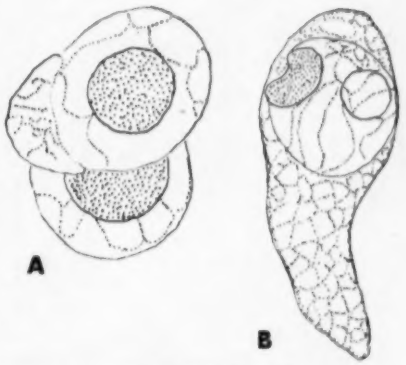
The first division of the apical cell is longitudinal, forming a two-celled true embryo (Figure 11 *B*). The plane of the next division is transverse and gives rise to a four-celled embryo (Figure 11 *C*). The plane of the third division is longitudinal and at right angles to that of the first. Thereafter cell divisions continue and differentiation of the embryo follows. Four days after pollination the embryos range from two to eight cells, at five days they range from eight to 32 cells, and after six days all are in excess of 32 cells.

The spherical embryo continues growth and cotyledonary initiation begins about nine days after pollination. The micropylar portion of the endosperm in the region of the embryo has by now become cellular, and cell formation is gradually extending chalazalward. The nucellus which was about 20 layers of cells in thickness is now reduced to 10 to 12 layers.

The cotyledons are in an early stage of development in the material collected 10 days after pollination. A marginal vacuole is present between the cotyledons and the adjacent cellular endosperm. The cellular endosperm fills most of the space previously occupied by the central lumen. A cone-shaped lumen persists between the cellular and chalazal free-nucleate endosperms. Further reduction of the nucellus is apparent at this time.

TABLE I. Totals and averages of seed classes and ovules dissected from 20 fruits collected at 10 days following pollination and from 50 fruits at maturity.

Classes	10 days		Maturity	
	Total	Av. per fruit	Total	Av. per fruit
Plump seed	62	3.1	147	2.9
Shranken seed	31	1.5	74	1.5
Total	93	4.6	221	4.4
Unfertilized ovules	217	10.9	Disintegrated	
Total	310	15.5		



FERTILIZATION
Figure 9

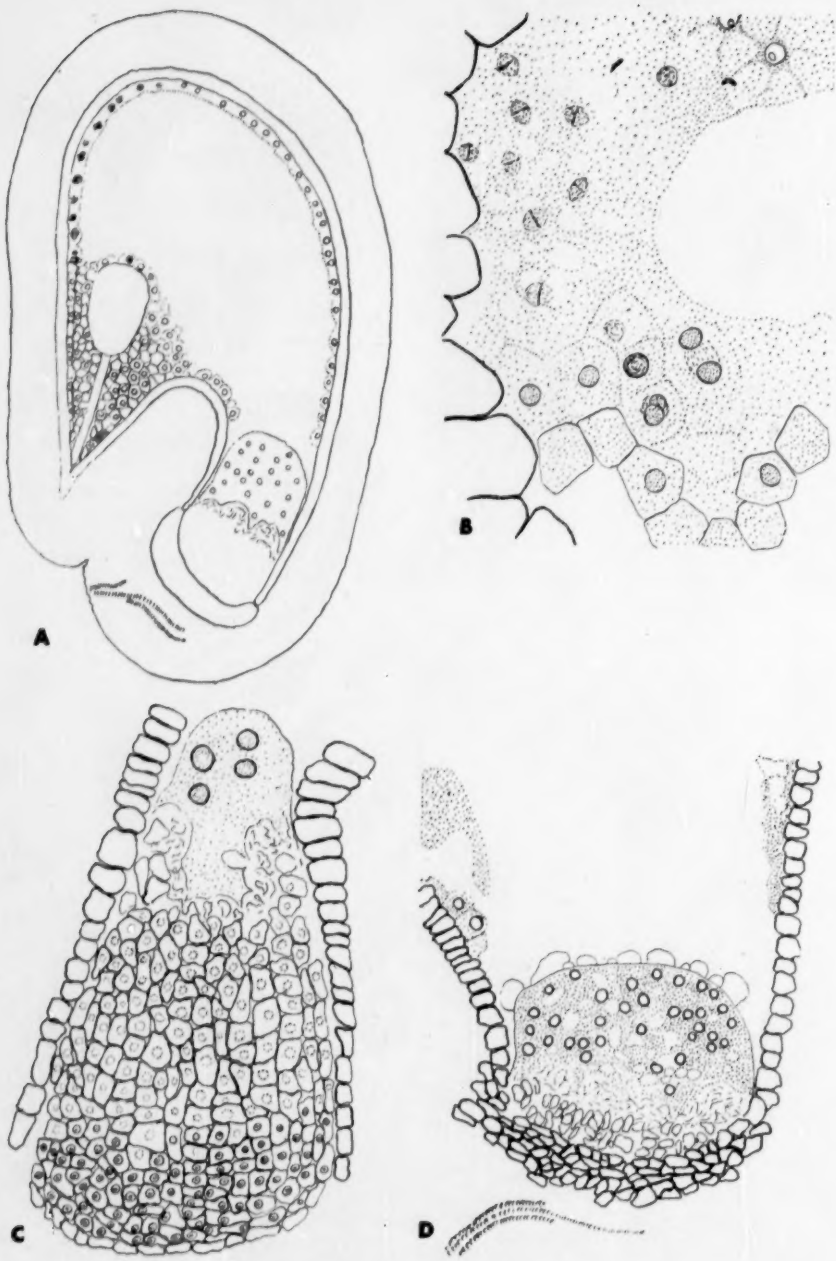
A—Fertilization of the polar nuclei. *B*—Fertilization of the egg. The polar nuclei and a male gamete fuse to form the primary endosperm nucleus. The egg and the other male gamete fuse to form the zygote.

The embryo is well differentiated by 18 days after pollination. The cotyledons are well formed but have not as yet reached the apex of the seed, although some curvature has taken place. The suspensor is in a state of collapse (Figure 11*D*). Cellular endosperm completely fills the central portion of the seed. The chalazal free-nucleate endosperm takes a much deeper stain than the cellular endosperm, indicating that storage materials are present. Hyaline globules move from the chalazal free-nucleate endosperm into the immediately adjacent conical lumen. The nucellus is being gradually consumed by the endosperm so that only five to seven layers of cells remain at this interval.

As growth continues the embryo becomes curved and gradually fills the space within the seed coat. There are from one to several layers of cellular endosperm adjacent to the inner wall of the seed coat. The chalazal endosperm continues to digest the nucellus so that at maturity little, if any, of this tissue remains. The gradually shrinking lumen becomes reduced to a small, partially conical space between the apices of the cotyledons, the chalazal free-nucleate endosperm, and the marginal cellular endosperm (Figure 11 *E*).

Seed failure following the cross C × P-7

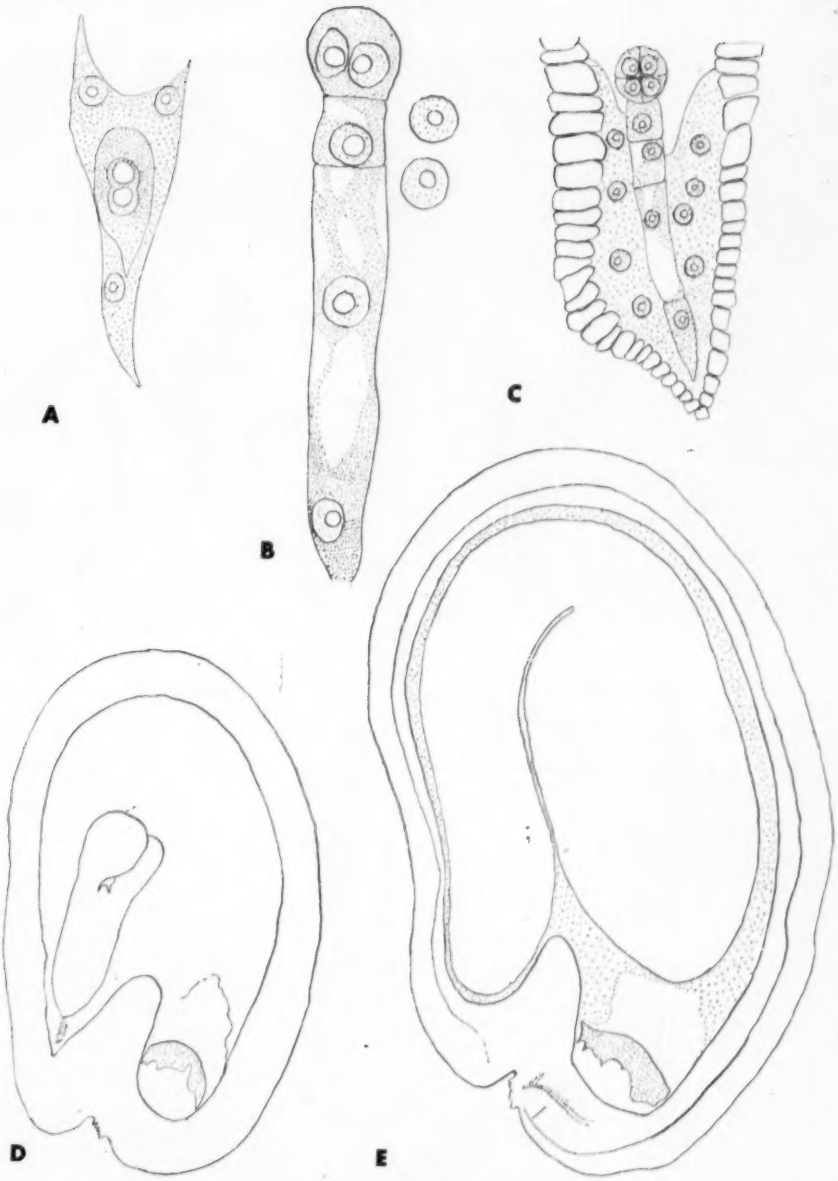
An abnormal relationship between the chalazal endosperm and the adjacent nucellus is the most frequent cause of seed failure. Failure of this type may occur during the earlier stages of development but is more pronounced



CELL WALL FORMATION

Figure 10

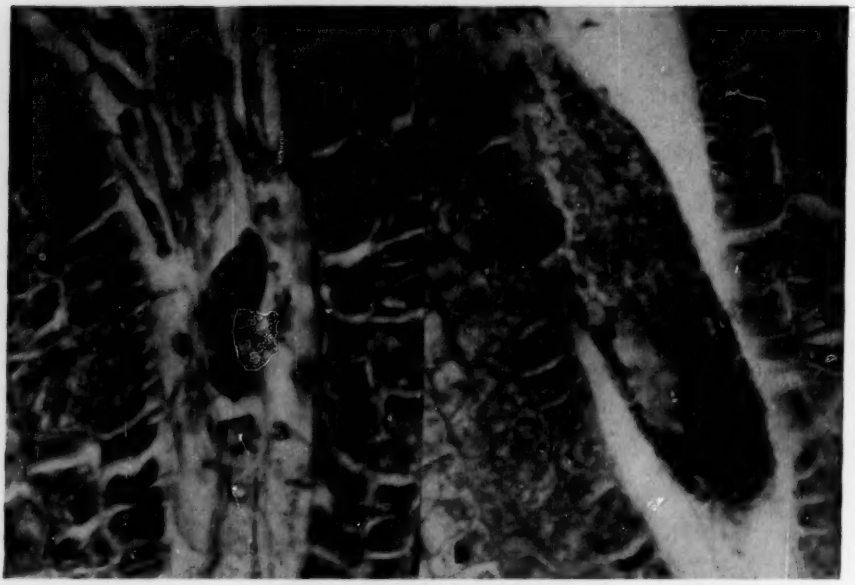
A and *B*—Cell wall formation in the endosperm follows the pattern of mitosis from the micropylar toward the chalazal end of the endosperm. *C*—High frequency of prophase nuclei in the nucellus. *D*—The nucellus is digested by the endosperm haustoria. Compare *C* and *D*.



LATER STAGES OF DEVELOPMENT

Figure 11

A—Division of the zygote. *B*—The two-celled true embryo. *C*—The four-celled true embryo. *D*—Collapse of the suspensor. *E*—Longitudinal section of the seed approaching maturity.



TRIPLE FUSION NUCELLUS

Figure 12

A—Normal triple fusion nucleus; *B*—Abnormal triple fusion nucleus. When the triple fusion nucleus fails to divide, it increases in size and the endosperm mother cell becomes filled with storage products.

at later stages. Small fragments of the nucellar tissue may be closely associated with the endosperm haustoria. In those seeds destined to fail, breakdown products are formed, possibly due to an upset in the physiologic balance between the endosperm and the adjacent nucellus. These products become more pronounced as development proceeds (compare Figure 7 *A* and *B*).

Seed failure due to embryo abortion occurs at a much lower frequency than that due to endosperm failure. If the endosperm continues to develop, abortion of the embryo early in ontogeny leads to germless seeds (Figure 8). The endosperm of such seeds becomes cellular two to three days earlier than it does in normal seeds.

Seed failure due to malfunction of the triple fusion nucleus may occasionally occur. Should the triple fusion nucleus fail to divide, it increases in size and the endosperm mother cell becomes filled with storage products (compare Figure 12 *A* and *B*). The embryo may develop for a short time but ultimately fails.

Relation between seed position in the fruit and development

The horseradish fruit is composed of two carpels separated by a partition. The somewhat flattened carpels dehisce along the two sides at maturity, exposing the seeds which remain attached to the vascular tissue at the periphery of the partition. The seeds are arranged in four rows, two on each side of the partition.

Fruits were collected 10 days after pollination and at maturity. Seeds were classified, using plumpness as a basis for separation. It was possible to distinguish the unfertilized ovules from the developing seeds at the 10-day interval, but impossible at maturity because the unfertilized ovules had distintegrated.

TABLE II. Position of seeds in twenty fruits collected at maturity. Position one is nearest the stylar end of the fruit.

Position	Mature Seeds																				Total	Av. seeds per fruit
1	1	1	2	2	1	2	2	1	1	1	1	1	1	0	1	0	1	2	2	1	26	1.3
2	2	1	1	0	1	2	1	0	2	1	1	0	1	2	2	1	0	1	1	1	21	1.0
3	2	1	1	1	1	1	1	2	1	1	1	1	0	2	2	1	1	0	1	0	25	1.3
4	1	1	1	1	2	1	0	1	0	0	1	1	1	1	0	1	1	1	2	1	18	0.9
Total	6	6	5	6	5	6	6	4	4	3	4	3	3	5	5	3	3	4	6	3	90	4.5

The data on developing seeds and ovules dissected from the fruits are summarized in Table I.

Ninety-three developing seeds and 217 unfertilized ovules were obtained from 20 fruits which were collected 10 days after pollination. Sixty-two of the 93 seeds were plump, whereas 31 were shrunken and in stages of collapse. The data on 50 mature fruits show approximately the same averages for plump and shrunken seeds as did the 10-day fruits.

Position of the ovule within the ovary was found to have little, if any, relation to the incidence of fertilization. Position one refers to the ovules nearest the style, while position four refers to the ovules nearest the receptacle. Twenty mature fruits were collected and the position of the seeds was recorded (Table II). There are four possible chances, on the average, for seeds to develop at each position. Since data from Table I show an average of 15.5 ovules per ovary, and in the latter study seeds were counted from stylar to the basal end, ovaries having less than 16 ovules would tend to lower the number of mature seeds in position four. The average number of seeds per fruit was found to be approximately the same for each of the four positions.

Discussion

Seed development in horseradish follows the normal cruciferous type similar to that occurring in cabbage.[5] Cross pollination is desirable for a good seed set in both species.

Fertilization occurs in about one-third of the ovules. It must be assumed that all functional ovules have the same chance to become fertilized, since position within the ovary had no effect on the frequency of fertilization.

Lack of fertilization has been shown by Cooper, Brink, and Albrecht[3] to be a common cause of reduced seed production in alfalfa. The apical ovules were more frequently fertilized than the basal ones. The fact that pollen tubes were shown to grow past the micropyles of some ovules suggests a physiologic disturbance.

Failure of the endosperm to function properly is the most frequent cause for seed failure in horseradish. Breakdown products are often present between the endosperm haustorium and the nucellus of seeds that are failing to develop in a normal manner. These products possibly result from a physiologic upset between the endosperm and the maternal tissue. Although endosperm-maternal tissue incompatibility has been reported in other plants, morphological symptoms differ. Brink[1] and Cooper[2] have shown that an overgrowth of the maternal tissue in the chalazal region is evidence of a physiological unbalance.

The only conclusive evidence of early embryo mortality noted in the present study is the formation of germless seed. Abnormalities in the endosperm are considered to be a major cause of seed failure. Since these abnormalities are more obvious than those in the embryo, it is not surprising that this should be the case.

Deleterious mutations affecting seed production might occur through long periods of clonal propagation. The Common horseradish clones used for commercial root production flower sparsely and produce no functional pollen. They are partially female fertile and produce a small quantity of seed when the proper male parent is used. Pound[4] has reported commercial horseradish to be 100 percent virus infected. Clonal lines that have become infected with the virus show characteristics similar to those of the Common horseradish under field conditions. The sparse flowering habit of these clones might possibly be due to the presence of the virus.

Summary

A histological study of seed development and failure in horseradish (*Amoracia rusticana* Gaertn.) is reported.

Fertilization occurs between 18 and 30 hours following cross-pollination.

About one-third of the ovules per ovary are fertilized.

Seed development follows the normal cruciferous type.

Lack of seed set is due to abortive ovules and failure of fertilization.

Seed failure is due to endosperm-maternal tissue incompatibility and embryo mortality.

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RULES FOR NOMENCLATURE IN TOMATO GENETICS

Including a List of Known Genes

D. W. Barton, L. Butler, J. A. Jenkins, C. M. Rick and P. A. Young*

INCREASING interest in genetic studies of the tomato has resulted in a rapid expansion of the list of known genes. The need for a standardized nomenclature is also evidenced by the very large number of workers who are conducting research in the genetics and breeding of tomatoes.

As one of its activities, the Tomato Genetics Cooperative has undertaken the responsibility of drawing up the following list of nomenclatorial rules, which have been under consideration by committees and the membership as a whole for the past two years. These rules have been frequently amended and revised in order to arrive at the simplest, yet most effective scheme of symbolization. Controversial aspects were settled by a vote of the membership.

In the form herewith presented, our list agrees with the corresponding "Rules for symbolizing genes and chromosome aberrations" compiled by a special committee of the International Union of Biological Sciences in response to a resolution of the Sixth International Congress of Genetics (Ithaca) that a standardized genetical symbolism be established.

Considering the unfortunate difficulties that have beset groups working with other organisms for which a single system had not been followed, we feel that the time is ripe for the establishment of a single standard in tomato genetics and therefore earnestly request that the following rules be observed.

Rules of Nomenclature

1. *Chromosomes.* The chromosomes are numbered according to their length as measured in pachytene. Such numbers have already been applied,[1] the longest being chromosome 1, the shortest, chromosome 12. In addition to length, such features as position of centromere, amount and distribution of heterochromatin serve to identify each chromosome.

2. *Linkage groups.* When the work of identifying linkage groups with chromosomes is sufficiently far advanced that all groups can be referred to definite chromosomes, the linkage groups will be renumbered so that the linkage groups bear the same numbers as their respective chromosomes. In the meantime it is suggested that linkage groups continue to be designated by Roman numerals and chromosomes by Arabic numbers. For the present, the term "chromosome linkage group" should be reserved only for those cases which have been definitely established. Thus, chromosome 2 and linkage group I are identical[1] and will be designated chromosome linkage group 2(I) when using the combined term, or simply as linkage group I when the chromosome is not mentioned.

3. *Genes.* Mutant genes are designated by letter symbols. The mutant name comprises an adjective or noun or a combination of both that refers to the main diagnostic feature of the phenotype. The initial letter of the symbol should be the same as that of the name; additional appropriate letters are added as necessary to distinguish it from other symbols already in use.

The genes *c, r, s,* and *y* do not conform to this rule, their symbols having unfortunately been derived from the normal instead of the mutant condition. Since these symbols have been in wide-

*Committees on Nomenclature and Gene List, Tomato Genetics Cooperative. Communications concerning this article and other affairs of the Tomato Genetics Cooperative should be directed to C. M. Rick, Chairman of the Coordinating Committee, T. G. C., Department of Vegetable Crops, University of California, Davis, California.

spread use for over 40 years, they should be retained, but all others should conform to this list of rules.

After obtaining reasonable evidence for the existence of a new gene for which the phenotype can be distinguished reasonably well in some or all genotypic milieux, the discoverer should select an appropriate name and symbol. Symbols that have already been reported should never be knowingly applied to other mutations.

4. *Alleles.* Dominance or recessiveness of a mutant gene is indicated by comparison with a "standard" or "normal" type. The variety Marglobe is proposed as this normal type since it is widely grown and is typical of the general concept of normal tomato morphology.

A mutant gene which is dominant to the normal type is written with the initial letter of the mutant name and symbol capitalized, while one which is recessive to the normal is written with all letters in lower case. The normal allele of a mutant gene is written with the symbol of the mutant gene followed by the superscript "+". Thus the normal allele of *sp* is sp^+ and of the mutant *I* is I^+. A dominant allele appearing later at the *sp* locus would be designated sp^D. Additional alleles at the same locus are designated by appropriate letter superscripts; thus for the *d* locus, the following alleles are known: *d*, $d^\times$, and d^+. When it is clear in the text which gene is concerned, the normal allele may be designated simply by the "+" symbol.

5. *Mimics.* When new mutants are found which are indistinguishable phenotypically from other previously described mutants, these may be designated by the name or symbol of the original mutant followed by a numerical subscript, the original mutant being assigned the subscript "1", as already applied in the case of male-sterile mutants, ms_1, ms_2, etc.

6. *Translocations* are designated by the symbol "T". The chromosomes involved in translocations are designated by their respective numbers. In order to distinguish between translocations involving the same chromosomes, lower-case letters are used following the chromosome number, thus T(1-2)a, T(1-2)b, etc.

7. *Inversions* are designated by the symbol "In" while the chromosome in which the inversion occurs is indicated by its respective number. Lower-case letters are used to distinguish different inversions on the same chromosome, thus, In(1)a, In(1)b, etc.

8. *Deficiencies* are designated by the symbol "Df" and are distinguished in the same manner as inversions (rule 7).

9. *Primary trisomics* are designated according to the extra chromosome present; thus, "triplo-1" refers to the primary trisomic of chromosome 1.

10. In order to distinguish between gene symbols and symbols of the chromosome aberrations, the former are italicized.

11. Since chromosomes of all investigated species of *Lycopersicon* appear to be almost completely homologous, it is suggested that the same symbolization apply to the entire genus. It is also proposed that the complete gene symbols not be duplicated among the species unless the genes in question are known to be identical, and the key letters of the symbols not be duplicated unless the genes are known to be allelic. Species alleles can be designated by a superscript to indicate the species, for example, a^h for a hypothetical allele in *L. hirsutum*.

List of Known Mutant Genes

The following table lists 107 gene symbols, their synonyms, main phenotypic characters, and references to pertinent literature. Symbols have been applied in accordance with the foregoing rules, and it is intended that they shall serve as a standard to avert duplication in future symbolization and to rectify faulty symbols that have been applied to certain genes in the past.

Symbols that duplicate certain ones previously applied or that pre-empt previously described genes, or whose corresponding gene has not been unequivocally established are given in parentheses.

Whenever the phenotype of a gene has been described in the summaries by Butler[3] or Young and MacArthur,[22] reference is made to those publications instead of the original de-

scription. A few of the more recently discovered genes have been described only in Reports of the Tomato Genetics Cooperative[21]; it is anticipated that these will be described ultimately in standard publications.

Symbol	Character	Reference
a (*a*$_1$)	*Anthocyaninless*; stems and leaves always lack purple color.	3, 22
ad	Resistance to *Alternaria* collar rot.	22
ag	*Anthocyanin gainer*; purple pigment appears on cotyledons and lower sides of leaves when growth is slow.	21
al (*a*$_2$)	*Anthocyanin loser*; purple stems become green in 10 to 21 days.	3, 22
an	*Anantha*; flowers greatly modified; inflorescences compound; closely resembles *ca.*	6
ap	*Apetalous* flowers; corolla reduced or absent; pollen scarce and nonfunctional.	17
as$_1$	*Asynaptic* meiosis; high pollen and ovule sterility.	20
as$_3$	*Asynaptic* meiosis; high pollen and ovule sterility.	20
as$_5$	*Asynaptic* meiosis; high pollen and ovule sterility.	20
as$_6$	*Asynaptic* meiosis; high pollen and ovule sterility.	20
as$_8$	*Asynaptic* meiosis; high pollen and ovule sterility.	20
at	*Apricot* or yellow-pink color of fruit flesh.	21
aw	*Without anthocyanin*; same phenotype as *a*.	5
B	High *β-carotene*; low lycopene.	11
bk	*Beaked*; fruits with sharp points at stylar end.	3, 22
br	*Brachytic*; internodes shorter than normal.	3, 22
bu	*Bushy*; internodes and inflorescences shortened; long petioles.	3, 22
c	*Potato leaf*; reduced number of leaf segments.	3, 22
ca	*Cauliflower*; extremely branched inflorescences, aborted flowers.	12
cb	*Cabbage*; large dark green leaves; fewer locules.	10
Cf$_1$(*Cf*$_{1a}$)	Resistance to Races 1 and 3 of *Cladosporium fulvum*.	3, 22
Cf$_2$(*Cf*$_{2t}$)	Immunity to Races 1 to 4 of *Cladosporium*.	3, 22
Cf$_3$(*Cf*$_{3t}$)	Resistance to Races 1 to 4 of *Cladosporium*.	3, 22
Cl$_1$	*Cleistogamous*; flowers fail to open.	17
cl$_2$	*Cleistogamous*; flowers open slightly.	17
d (*d*$_1$)	*Dwarf*; all parts foreshortened; leaves dark and rugose.	3, 22
d^x	*Extreme dwarf*; recessive to *d* and *d*^{s}.	21
dl	*Dialytic*; stamens not united; hairs partially suppressed.	3
dm (*d*$_2$)	*Dwarf modifier*; with *d* causes extreme dwarfing.	3
dv	*Dwarf virescent*; pale green at growing point, changes quickly to normal green; plants always stunted.	18
e (*b*)	*Entire*; fewer leaf segments and distorted midvein.	3, 22
el (*e*)	*Elongated* fruits as in Oxheart.	3
ex	*Exserted* stigmas, or styles twisted within anther tubes.	17
f	*Fasciated*; many-loculed fruits as in Ponderosa.	3, 22
fl	*Fleshy calyx*; sepals often curled.	3
(*G*)	Inhibits modifiers, permitting expression of *r*.	22
g	*Grooved* fruits; may be associated with fasciation.	23
gs	*Green stripes* in epidermis of unripe fruit, golden in ripe fruit.	21
H	*Hairs* absent except on hypocotyl and at growing point.	3, 22
hl	*Hairless*; no hairs on hypocotyl or rest of plant.	3
I	*Immunity* to race 1 of *Fusarium lycopersici*.	22
j	*Jointless* pedicels.	3, 22
(*K*)	Inhibits modifiers; permitting expression of *t*.	22
l	*Lutescent*; yellowish unripe fruits; premature yellowing of leaves.	3, 22
Lc	*Locule* number reduced; associated with *o*.	3, 22
(*lc*$_1$,*lc*$_3$,*lc*$_5$)	Control *locule* number.	13
lf	*Leafy*; indeterminate inflorescences.	3, 22
lg	*Light green* foliage.	21
m	*Mottled*; leaves and cotyledons with flecks of paler green.	3, 22
mc	*Macrocalyx*; sepals leaf-like.	3, 22
	(The 18 *ms* mutants are *male-sterile* in the respect that they deviate from normal only in the absence or extreme scarcity of functional pollen; parent variety is stated).	
ms$_1$	Pale, shrunken anthers; no pollen; hybrid stock.	14
ms$_2$	Pale, shrunken anthers; no pollen; Pearson.	14
ms$_3$	Very pale, shrunken anthers; collapsed pollen mother cells; San Marzano.	14

Symbol	Character	Reference
ms_4	Pale, shrunken anthers; a few aborted pollen grains; Early Santa Clara.	14
ms_5	Abnormally small flowers; very pale and greatly shrunken anthers; usually no pollen; San Marzano.	15
ms_6	Shrunken, pale anthers; no pollen; San Marzano.	15
ms_7	Nearly normal-colored, slightly shrunken anthers; aborted pollen in tetrads; San Marzano.	15
ms_8	Abnormally small flowers with exserted stigmas; pale shrunken anthers; no pollen; San Marzano.	15
ms_9	Anthers nearly normal; no pollen; San Marzano.	15
ms_{10}	Abnormally small flowers; small, very pale anthers; greatly exserted stigmas; no pollen; San Marzano.	15
ms_{11}	Very pale, shrunken anthers; aborted pollen, free or in tetrads; San Marzano.	15
ms_{12}	Abnormally small flowers; nearly normal-colored but shrunken anthers; no pollen; San Marzano.	15
ms_{13}	Nearly normal anthers; free aborted pollen; San Marzano.	15
ms_{14}	Very pale, shrunken anthers; aborted pollen; Earliana.	21
ms_{15}	Abnormally small flowers with exserted pistils; very pale, dwarfed anthers; no pollen; San Marzano.	21
ms_{16}	Pale, shrunken anthers; clumped aborted pollen; Pritchard.	21
ms_{17}	Pale, shrunken anthers; no pollen; Ace.	21
ms_{18}	Exserted stigmas; slightly pale, very shrunken anthers; no pollen; Cal-255.	21
mt	*Midget*; all parts of plant reduced; high sterility.	9
n (*nt*)	*Nipple tips* at stylar end of fruits.	22
nc	*Narrow cotyledons*; slow growth.	3
ne	*Necrotic*; with Cf_2 causes progressive necrosis of leaves.	3, 22
o	*Ovate* or pear-shaped fruits.	3, 22
(*O*, *O'*, *o*)	Spherical, oblate, and elongate fruits.	23
ol	*Ovate*; elongate fruits with low locule number.	5
p	*Peach*; dullness and increased hairiness of fruit epidermis.	3, 22
pc	Sticky fruit *epidermis*.	3
pi	*Ristillate*; flowers usually lack stamens and are otherwise modified.	17
pr	*Propeller-like* cotyledons; true leaves greatly modified.	4
ps (*va*)	*Positional-sterile*; corolla does not unfurl; pollen is not shed.	8
r	Yellow color of fruit flesh.	3, 22
rc	*Rolled cotyledons*.	22
ri	*Ridged* or rough leaves.	22
ro	*Rosette*; extremely short internodes; no flowers.	4
rv	*Reticulate virescent*; cotyledons and new leaves pale with dark veins, turning to normal green with age.	18
s	Compound inflorescence; greatly increased number of flowers.	3
Se	*Septoria* resistance	22
sl	*Stamenless*; stamens usually absent; modified fruit form.	2
Sm	*Stemphylium* resistance	7
sp	*Self-pruning;* determinate plant habit	3, 22
st	*Sterile*; completely sterile; plants later become purple.	22
t	*Tangerine*; orange color of fruit flesh and stamens.	3, 22
tf (*cf*)	*Trifoliate*; leaf with usually only three segments and long petiole.	18
u (u_1)	*Uniform*; unripe fruits of uniform light green color without usual darker shoulders.	3, 22
ug (u_g)	*Uniform gray-green*; as of *u* except for grayish color.	3, 22
v	*Virescent;* white seedlings	22
Ve	*Verticillium* resistance.	19
vg	*Vegetative*; greatly deformed, usually functionless flowers.	17
vi	*Villous*; stems very hairy.	21
w	*Wiry*; slender, thread-like leaflets; dwarfed plants.	3, 22
wd	*Wilty dwarf*; grayish-green, droopy leaves; stunted plants.	16
wf	*White flower*; corolla color is buff, light tan, or white.	3, 22
Wo	*Woolly*; all parts densely pubescent; homozygous lethal.	3, 22
wt	*Wilty*; leaf margins curl adaxially.	3, 22
x	Ineffective microgametes associated with *I*.	22
Xa	*Xanthophyllic*; yellow leaves; retarded growth; homozygous lethal.	3, 22
y	Colorless fruit epidermis.	3, 22

Symbol	Character	Reference
ys	*Yellow seedlings*; lethal in early stage.	3, 22
yv	*Yellow virescent*; new foliage is pale yellow-green; turning to normal green with age.	18

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DUCKY, A NEW SECOND CHROMOSOME MUTATION IN THE MOUSE

GEORGE D. SNELL*

IN 1946 some mice with a peculiar, waddling gait appeared in a non-inbred ruby silver stock. Investigation has shown this trait to be a recessive Mendelian character linked with dilution and short-ear. We have called it "ducky" (symbol *du*).

The ducky stock at the Jackson Laboratory was entirely wiped out at the time of the Bar Harbor fire. Fortunately some homozygous ducky mice had been sent to Dr. Clyde Keeler, and one of these, a male, which he kindly returned, proved to be fertile. Our present stock was recovered from this one animal.

Description

The abnormal condition may begin to show up any time after the mice reach 14 days of age but is seldom clear before 19 days. In a few cases we have not been able to make a final classification before 40 days.

The first expression is usually a slightly hunched appearance and a toeing out of the hind feet. In more advanced stages there is a waddling or reeling gait and a tendency to fall to one side (Figure 13). In older animals the toeing out usually disappears, but the reeling and falling become more pronounced, the gait suggesting intoxication. In some partly grown mice the first manifestation noted may be a jumpiness and an absence of coordinated running. In such cases, however, the reeling and falling appear after the excitement of a few minutes of handling.

Affected mice are slightly smaller than their normal sibs and their viability is reduced. Some of the mortality in ducky mice over 14 days of age may be due to difficulty in obtaining food and water. In raising them we have found it desirable to place rolled oats or other soft food directly in the box, and to make sure that the spout of the water bottle is within easy reach. One male, without special care after growth was completed, lived to be 16½ months old. This is the longest survival so far noted.

Hearing is apparently normal.

The majority of males that reach maturity are fertile, but rather poor breeders. Eight litters is the most we have obtained from one male. Of several females raised to maturity only one bred. She gave a single litter by a ducky male.

The physiological or anatomical basis for the trait is unknown.

Genetics

The one mating of ducky × ducky yielded a litter of eight young which were given to a foster mother. The four young that survived were all ducky.

The mating ♀ *Dudu* × ♂ *dudu* gave 85 young of which 66 lived long enough to be classified. Of these 66, 36 were normal and 30 ducky.

The ratio from the mating *Dudu* × *Dudu* has shown considerable variation, probably conditioned by various factors affecting the health of the colony or of individual litters, and by the age at classification. In the mating involving dilution (Table I), in which classification was generally made at optimum age, the ratio was 115 normal to 22 ducky.

The deficiency of ducky individuals as compared with the expected 1:1 and 3:1 ratios is probably due entirely to differential mortality rather than to the occurrence of normal overlaps. Evidence that this is the case is provided by (1) the absence of normals in the one litter from ducky × ducky, (2) the clear expression of the trait in all individuals raised to maturity, and (3) the failure among many mice raised from the ducky stock, to find any individuals which were phenotypically normal but which bred like homozygous duckys.

We have occasionally noted individuals which showed some toeing out at about 19 days but which later turned out to be normal. It is possible that these were heterozygous for

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This investigation has been aided by a research grant C-1329 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

ducky and that the gene *du* is therefore partly dominant, but we have not tried to check this supposition by a breeding test.

Because of a slight resemblance of ducky to jittery[1] and reeler,[2] ducky was tested for identity or non-identity with these two traits. The mating *DuduJJ* × *DuDuJj*, gave 20 normals; the mating *duduJJ* × *DuDuJj*, gave eight normals, and the mating *duduRlRl* × *DuDuRlrl* gave 21 normals. This is evidence that ducky is not allelic with either of these two genes. Further evidence is provided by the linkage tests described below.

Linkage Tests

Linkage tests were set up with ducky, using standard linkage stocks maintained at the Jackson Memorial Laboratory.[4] Twenty-one loci were included in the tests (or 22 counting the two closely linked loci *d* and *se*), namely, *a, b, c, Ca, d, f, Fu, fz, je, ln, p, Re, ru, s, sh-2, Sp, v, W, wa-1, wa-2* and *Wh*. The tests revealed clear linkage with *d* and *se*, in linkage group II, and probable absence of linkage with all the other genes.

Two separate tests were run involving dilution (*d*), only one of which also included short-ear (*se*). In the test involving *d* alone, there were no crossovers in 137 F_2 mice, (observed ratio: ++:*du*:*d*+:*ddu*::76:22:39:0). The results from the cross involving all three genes, *d*, *se* and *du*, are shown in Table I. In the case of this test it was unfortunately impossible to have all the animals classified by one person, and there may have been some faulty classifications as a result, both with respect to ducky and dilution. Classification of dilution was complicated by the fact that several other color genes were segregating in the same cross. Animals regarded as possibly doubtful are indicated in Table I with parentheses and a question mark. There was one crossover of the genotype *dsedu* which was adequately checked; also one individual of the genotype +*se*+, and hence a crossover between *d* and *se*, which can be accepted as established. Combining all data, but treating only one individual as a crossover, the observed ratio becomes ++:*d*+:+*du*:*ddu*::179:88:59:1, and the crossover percent, calculated by the method of Finney,[3] 12.4 ± 5.5. If all possible *ddu* individuals are classed as such, the ratio is ++:*d*+:+*du*:*ddu*::179:85:56:7, and the crossover percent 31.2 ± 5.0.

TABLE I. Linkage of dilution, short ear and ducky. F_2 data, where initial cross was ♀ P (*aabbdse/dsepprr*) × ♂ *dudu* (*Cc*).

No. of mice when first recorded	*se*	+ + +	+ + *d*	*s* + +	*d* + +	*dse* +	*d* + *du*	*sedu* +	*dudu*	Total survivors
244	7	102	11	1	14	45	31	0	4(3?)	197

DUCKY MICE, SHOWING ABNORMAL BEHAVIOR

Figure 13

Two ducky mice, aged 26 days. One shows the toeing out of the hind feet, the other has fallen over on the right side.

The former value probably more nearly represents the true value. In either case the existence of a linkage is unquestionable.

The one individual of the genotype +*se*+, representing a crossover between *d* and *se*, with *du* staying with *se*, would suggest that the order of the genes is *d se du*. The formula of the F_1 mice would then be *dse*+/++*du*. More evidence is needed, however, before this order can be regarded as established.

The results with most of the other genes tested gave no evidence of linkage. In the case of three loci other than *d*, however, a possibility of linkage exists. These are *c* (45.7 ± 2.9 percent crossing over), *p* (43.3 ± 5.6 percent crossing over), and *f* (41.2 ± 3.6 percent crossing over). The significance of the

results in these three cases is somewhat diminished by the disturbed ratios. This is particularly true in the case of *f* or flexed tail, where there was not only a deficiency of *du*, but also an even larger deficiency of *f*. This is a common occurrence with this gene where classification is not made at birth on the basis of the anemia which accompanies it. Most of our animals were classified at weaning on the basis of the flexed tail or belly-spot which are the other two manifestations of the gene, and both these characteristics show frequent normal overlapping.

Hence while there is a possibility that future linkage tests will show *f* to belong in linkage group II, or else groups I and II actually to be identical, we are inclined to regard the apparent linkages other than that of *d* and *du* as the product of accidents of sampling.

Summary

Ducky (symbol *du*) is a new recessive mutation in the mouse causing abnormalities of behavior beginning at about 19 days of age. In affected individuals there is a toeing out of the hind feet, and, as they get older, a reeling gait with a tendency to fall to one side. There is reduced viability. Males show a limited fertility; only one female so far has bred.

Ducky is linked with dilution and short-ear.

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PHYLOGENY AND MORPHOGENESIS

THE underlying idea of this book* is stated thus on the fly leaf: "Morphogenesis; which is considered in detail in this volume, would appear to provide as much as any other contemporary theme, a suitable focal point for what the author believes may be the onset of the golden age of botany. But no matter what plant is selected for experimental investigations, that plant has, in its genetic constitution, a vital link with the past. Phylogeny and morphogenesis are not separate disciplines: they are one." It seems that the author, as a morphologist, felt that the modern disciplines of genetics, plant physiology and experimental morphology tend to forget that plants are also as Theodor Boveri expressed it in a classic oration, "historical beings." This means, of course, that certain features of an organism cannot be understood completely by experimental analysis, because there is a residuum which is understandable only on the basis of the phylogenetic history. Thus the author wants to integrate phylogeny and evolution with morphogenesis, physiological genetics and physiology of form and growth. In doing so he draws also considerably on zoological material, which is understandable in view of the surprising backwardness of experimental embryology of plants as compared with the great successes of this science in animals.

In pursuit of such a goal the author has collected and assembled a large body of facts from the diverse fields of plant sciences. But the reviewer feels that these facts are marshalled as a kind of program indicating the aspects of the problems and the relevant material, rather than integrating them into a whole. It is possible that such an integration is not yet possible, or, that it requires more hypothesizing than the author is ready to do. But the effect is that the individual parts of the book, interesting and stimulating as they are, appear as more or less isolated units. It is possible that the author renounced, out of a certain modesty, a synopsis, in the literal meaning of this Greek word and felt content to assemble the material for a future synopsis. As such an assemblage the book is very interesting, and animal—as well as plant—geneticists and evolutionists will derive profit from its study.

The first part of the book deals in a very special way with the phylogeny of the ferns in which Bower's work is put in the foreground. The great lines of this phylogeny are indeed very interesting to the evolutionist and deserve to be analyzed with the present-day evolutionary ideas (including those of the reviewer) in mind. But the author while presenting Bower's Lamarckian and even mnemistic explanations with only slight censure, expressly refuses a modern type of analysis. Thus it is not clear why he wants to present

(*Continued on page* 34)

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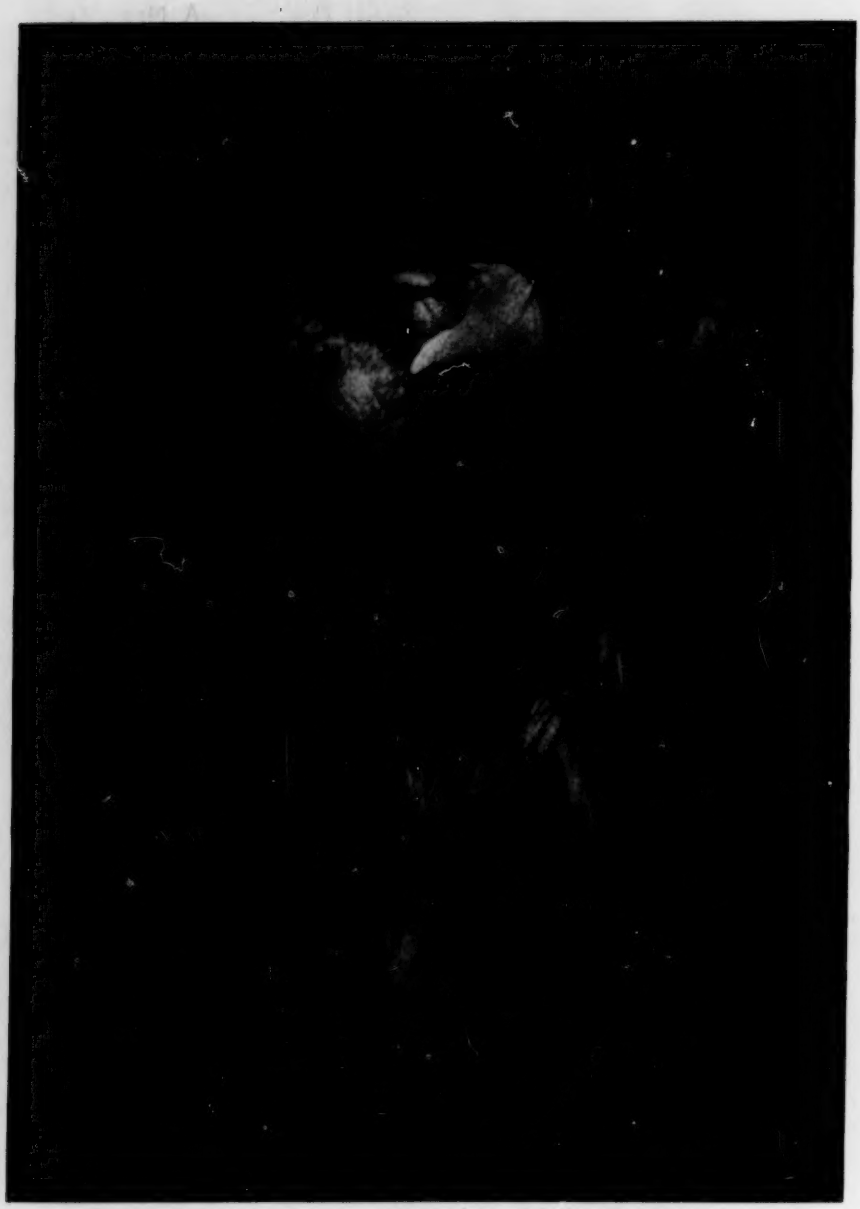


ALOPECIA

Figure 14

Homozygous alopecia mice at about one month of age. With the exception of the guard hairs, all hair is lost at this age.

ALOPECIA, A DOMINANT MUTATION IN THE HOUSE MOUSE

MARGARET M. DICKIE*

THIS report describes a new mutation that affects the deposition of the normal hair coat of mice. Such genes as hairless *hr*, rhino hr^{rh} (hypotrichosis cystica) and naked *N* (hypotrichosis hypokeratotica) affect the manner in which the hairs develop and moult. Following the first moult, a normal coat is never seen again in mice of these genotypes.[1] This new mutation, alopecia, behaves somewhat similarly to naked.

Origin and Breeding Data

The first animals in which abnormal hair coats occurred were F_1 offspring of a C57BL/10 female mated with a fuzzy male (one of the first offspring of the fuzzy stock which Dr. Clara Lynch returned to this laboratory following the fire of 1947). Abnormal coats of hair were found among the offspring of these F_1 animals mated *inter se* and among offspring of the F_1 animals backcrossed to the fuzzy male.

Alopecia seems to be semi-dominant since the abnormal coated animals were found in the F_1 generation and both heterozygous and homozygous animals seem to be distinguishable. *Al* is used as the symbol for this character. When alopecia animals were mated to C3H/Di animals, the F_1 generation produced either 53.33 percent or 100 percent alopecia offspring, depending upon the genotype of the alopecia animal that was used in the cross (Table I). Heterozygous animals mated *inter se* produced 57.53 percent alopecia, a deficiency as compared with the expected 75 percent. Homozygous alopecias mated *inter se* produced 100 percent alopecia offspring.

Description

Alopecia is first noticeable when animals are 25 to 32 days of age. At this time it is possible to distinguish *Al/Al* animals from *Al/+* animals. *Al/+* animals lose all but the guard hairs on the ventral surface and the rest of the coat is not quite as smooth as the coat of a normal animal of the same age. *Al/Al* animals at this time lose all hair over the entire body with the exception of the guard hairs (Figure 14). After the moult the other hairs are replaced and the coat appears almost normal. Following loss of hair at each successive moult, fewer hairs seem to be replaced and the animals have a very patchy appearance. No histological studies have been done on the skins of the animals but hair types have been studied. At weaning only guard hairs or awl type are present. Between moults all normal hair types are found.

Genetic Studies

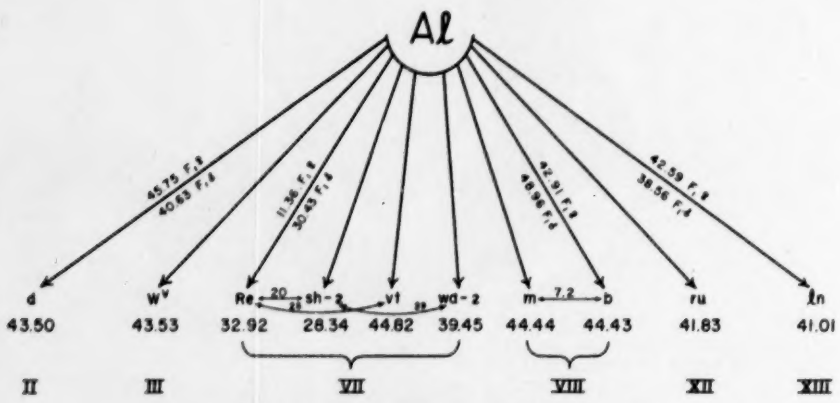
Linkage studies have been carried out with the genes of various linkage groups and several facts can now be reported. The data are summarized in Table II. All linkages were tested by means of backcross coupling and backcross repulsion matings.

Alopecia gave significantly less than 50 percent recombination with several different genes belonging to the various linkage groups. It was apparently linked with dilution (*d*) on linkage group II; for 439 animals the recombination value was 43.50 percent. Offspring of F_1 females showed 45.75 percent recombination and of F_1 males, 40.63 percent. When only non-alopecia classes were tabulated the recombination percent was 43.95.

Dominant spotting (W^v) on linkage group III gave an apparent recombination percent of 43.53. All animals classified were offspring of heterozygous females.

Linkage was apparent with four genes belonging to linkage group VII. In this group, rex (*Re*) is 20 units from shaker-2 (*sh-2*) and 25.4 units from vestigial tail (*vt*). Shaker-2 is 29 units from waved-2 (*wa-2*). Rex and alopecia showed a crossover percent of 26.92 in 182 animals; with females as the heterozygous parent, the crossover percent was 11.36

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This work is supported by research grant C-362 to the Roscoe B. Jackson Memorial Laboratory from the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare.



LINKAGE RELATIONSHIPS OF ALOPECIA

Figure 15

Schematic presentation of apparent linkages of *Al* with genes on known linkage groups.

and with males as the heterozygous parent, 30.43 percent. In a new test with rex, the crossover percent was 45.10 for 87 animals. Combining all data, the crossover percent was 32.92. Shaker-2 and waved-2 were tested simultaneously and heterozygous parents were all females. For 441 animals classified, the crossover percent with shaker-2 was 28.32 and with waved-2, 39.45 percent. Data on 87 animals classified for vestigial tail and alopecia gave a crossover percent of 44.82.

Data on linkage with brown (*b*), linkage group VIII, were obtained from a cross with misty brown animals and from a cross with dilute brown animals of the DBA/2Wy strain. The crossover percent for misty and alopecia, using only F_1 females was 44.44 and for brown 41.97 percent. The linkage with dilution has already been mentioned; with brown in the same 439 animals, the crossover percent becomes 44.43. F_1 females produced 406 offspring with 42.29 percent crossovers and F_1 males, 192 offspring with 48.96 percent crossovers.

When considering only the non-alopecia classes for these three genes, the crossover percentages are 52.52 with misty, 45.42 with brown and 43.95 with dilution. Such manipulation of the data would eliminate linkage with misty and perhaps with brown, but not with dilution.

On linkage group XII ruby (*ru*) showed a recombination of 35.48 percent for 248 offspring from heterozygous females. A new test of this linkage was made and the recombination value was 49.24 percent which would indicate there was no linkage. When all data were combined, however, the recombination value was 41.83 percent.

Alopecia also appears to be linked with leaden on linkage group XIII. In the first test in which the leaden gene was obtained from a cross to the V stock, 252 offspring from F_1 females of this cross gave 40.48 percent recombination. The second test employed the leaden gene obtained from a cross to strain C57L/Fe. Three hundred eighty-two offspring gave a recombination percent of 41.09. The source of the leaden gene made no difference to the recombination percent. Combination of all data gave 634 animals with recombination of 41.01 percent. When only non-alopecia classes were tabulated, the recombination percent for 462 animals was 42.42.

This apparent linkage with leaden was peculiar since tests with fuzzy and splotch, both of which are linked with leaden[3] were negative or over 50 percent.

In four of the linkage tests—all backcross repulsion type—there was an excess of alopecia animals, but in all the other tests there was a large deficiency of alopecia animals.

Discussion

The accumulation, tabulation and presentation of such a large amount of data needs some clarification and explanation. Data were accumulated in large numbers because of the apparent miscellaneous linkages. However, the linkages did not disappear when more animals were classified. Although there may have been some error in classification of *Al*/+ animals in those tests where loose linkages were apparent, the animals were reclassified a week later to reduce any such error and the tabulation of non-alopecia data follows the same pattern as the total data.

Not only are the linkages dispersed over the linkage group map (Figure 15), in complete

disagreement with the linkage relationships already established for genes in the known linkage groups, but also in group XIII there is linkage with only one gene, while on group VII alopecia is apparently linked with four genes. There is no possible linear arrangement of the genes on either VII or XIII when alopecia is included. This disparity prevents linkage of these groups with one another for the same reason. There seems to be no linkage relationship with sex although there is a deficiency of recombination with rex on linkage group VII. It has been suggested by Fisher that such deficiencies may occur since VII might be the homologous arm of the sex chromosome.[1]

Several possible explanations for the multilinkage phenomenon of alopecia may be mentioned. The background or strain upon which the genes used for the linkage tests were placed might have an influence on the expression of the gene. Alopecia has not been made isogenic with any inbred strain but has been maintained by brother by sister matings since the appearance of the mutation. Consequently in this stock the gene might not find as full an expresison as it might on an inbred strain background. In linkage crosses, some of the genes tested were isogenic with certain inbred strains and these strains may have interfered with the expression of alopecia. Tests of alopecia on different backgrounds are being carried out to determine this.

Recently Michie[3] has advanced a theory of affinity whereby the centromeres of different chromosomes may show non-random segregation so that apparent linkages of genes near these centromeres are revealed. However, they do not show proper linear arrangement of linked loci on that chromosome.

In support of the theory of affinity Wallace[8] has given a case where short-Danforth (*Sd*) and caracul (*Ca*) appeared linked in one test but in other data the independence of the two genes has been well established.

If such a phenomenon as affinity is in operation, it would seem that the various quasi-linkages of alopecia with the genes on different linkage groups is a striking example of affinity. Alopecia could be said to be located near the centromere of a linkage group, but from the data herewith presented, it appears to be on a linkage group with no other marked loci and all apparent linkages shown here are the affinity linkages and do not establish the location of the alopecia gene.

Summary

A new mutation named alopecia which affects the formation of a normal coat of hair in mice is described. The alopecia mice regularly lose all but the guard hairs at each moult. The character seems to be semi-dominant since heterozygous and homozygous animals can be distinguished. *Al* is suggested as the symbol for the character. Linkage studies with alopecia show apparent linkage with the follow-

TABLE I. Breeding data

Type of Cross	*AlAl*	*Al+*	*++*	%*Al*
AlAl × *alal* (C3H)	0	141	0	100
AlAl × *AlAl*	216	0	0	100
Alal × *Alal*	47	60	79	57.51
Alal × *alal* (C3H)	0	40	35	53.33

TABLE II. Results of linkage studies

			Heterozygous ♀♀					Heterozygous ♂♂					
Al tested with	Linkage Group	Mode of Test	*AlX*	*Alx*	*+X*	*+x*	Recomb. %	*AlX*	*Alx*	*+X*	*+x*	Recomb. %	Total Recomb. %
d	II	BC_c	50	46	67	84	45.75	45	25	53	69	40.63	43.50
W°	III	BC_c	50	78	18	24	43.53						
t	III	BC_c	35	35	41	42	49.67						
Sd	V	BC_c	6	8	13	19	54.34	7	14	15	20	48.21	50.98
Ca	VI	BC_c	—	—	—	—		76	69	20	30	54.36	
rh-2	VII	BC_c	131	38	87	185	28.34						
wa-2	VII	BC_c	113	56	118	154	39.45						
Ro	VII	BC_c	2	22	17	3	11.36	15	64	32	27	30.43	26.92
		BC_c	13	7	21	10	45.10						32.92
vt	VII	BC_c	21	13	26	27	44.82						
b	VIII	BC_c	46	17	51	48	41.97						
			50	46	59	92	42.91	35	35	59	63	48.96	44.41
m	VIII	BC_c	43	20	52	47	44.44						
Fu	IX	BC_c	60	62	14	26	53.08						
v	X	BC_c	32	35	51	31	57.71						
ru	XII	BC_c	54	22	66	106	35.48						
			42	46	52	59	49.24						41.83
ja	XII	BC_c	38	30	78	79	48.00						
In	XIII	BC_c	66	43	59	84	40.48						
			27	13	99	124	42.59	15	8	38	58	38.65	41.01
fu	XIII	BC_c	12	12	38	38	50.00	25	19	29	19	52.17	51.04
Bp	XIII	BC_c	9	15	8	12	47.73	47	48	24	40	54.72	53.20
f	XIV	BC_c	27	24	49	31	55.72						

ing: dilution, dominant spotting, shaker-2, rex, vestigial tail, waved-2, misty, brown, ruby and leaden. Two possible explanations are discussed to try to clarify this multi-linkage data. They are the possible effect of the background of the stocks employed on the expression of the gene and the possibility that Michie's principle of affinity may be operating and masking the true location of the alopecia gene.

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Phylogeny and Morphogenesis

(*Continued from page 29*)

phylogenetic work in its old fashioned way, instead of telling us how to integrate it into present-day work. Also the following two chapters stand more or less alone and are possibly meant as a historical backdrop to what follows. There are actually 125 pages of these introductory chapters, the relations of which, to the rest of the book, are very tenuous.

The actual work (as the reviewer sees it) begins with the 8th chapter on morphogenesis and causal morphology. After a discussion of some general features of causal morphogenesis in plants, emphasizing its physiological background and leading to a cautious but sympathetic discussion of Smut's holism, genetics, especially physiological genetics are introduced. Action and nature of genes, biochemical genetics, cytoplasmic inheritance are shortly surveyed with the idea that here the physiological processes underlying morphogenesis are revealed. This includes also the work on polyploidy and form and especially genetic constitution in relation to shape, size and differentiation. The 12th chapter on biochemistry and morphogenesis is one of the central chapters, containing a rather elaborate review of the relations of phytohormones to morphogenesis. The different approach of animal causal embryology and the reasons for it are emphasized. A special discussion is devoted to polarity. Again the reviewer misses the integration of the facts with those of genetics and physiological genetics. It seems that the author wanted more to interest the plant morphologist in the different aspects of work with which he is less acquainted, but did not consider it his duty to perform a synthesis. The next chapters go into details of plant morphogenesis and organ formation. Their excellent illustration and rich content indicate the author's personal interest in the subject. To the zoological geneticist these are the most interesting chapters, actually the nucleus of the book because they summarize much important work of present-day plant physiology which has a great bearing on the understanding of morphogenesis and with it genic action in a biochemical way. Though many of the principles at work in plants will be found to have only a small counterpart in animals the physiological geneticist will have to watch this work, which includes also tissue culture and histogenesis.

The physical factors of form, including the mathematical treatment, are the next topic, clearly influenced by D'Arcy Thompson's brilliant volume. The general conclusion is reached that though the primary genetic actions in the cell are biochemical, simultaneously or as a direct consequence, physical factors become incident, which largely determine form and structure.

Under the title of experimental morphology again a specific botanical chapter follows, in which the author delves with obvious special interest into the problems of the shoot apex, followed by a detailed examination of comparative organization in pteridophytes and flowering plants, leaf organization, leaf order and similar problems. This is again a chapter recommended to the physiological geneticist of animals, as it brings out the great difference in plant organization and, with it, the necessity for a different experimental approach, perhaps also an explanation for the little progress of physiological genetics in plants as compared with that of animals. Finally a short chapter deals with the theory of organization in plants and tries to look for links with theories of animal organization and development.

The book winds up with an attempt at synthesis which summarizes the major chapters. The reviewer already indicated that he would have preferred the synthesis worked into the whole of the book. Altogether this is a very interesting and worthwhile book and especially remarkable because it tries to link morphology with physiology and genetics with equal emphasis upon both kingdoms. An extensive bibliography and a good index are added.

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AN UNUSUAL ASSOCIATION OF TWO MUTANT CHARACTERS

In Atom-Bombed Barley

C. C. Moh, R. A. Nilan and F. C. Elliott*

During the analysis of barley mutants induced by atomic bomb- and X-radiation a particular line was found which produced an unusually high proportion of yellow-green seedlings following self-fertilization of green, partially sterile plants.[4] Among the green plants which appeared in the perpetuation of the mutant line there was segregation for fertility and partial sterility, but yellow-green plants were always fertile. This paper is concerned with the nature and association of yellow-green seedlings and gamete abortion in this mutant line of barley.

Materials and Methods

The mutant line arose in a population of six-rowed barley (*Hordeum vulgare* var. Trebi, $2n = 14$) which was subjected to atomic-bomb radiation at Bikini in 1946. The X_2 to X_7 generations were grown at Pullman, Washington.

Progeny tests were conducted in the greenhouse. The numbers of yellow-green and green seedlings were recorded within each plant progeny when the first leaves of the seedlings were fully developed (about 12 cm. high). Since the yellow-green plants were viable, it was possible to test for gamete abortion in these plants.

Partially sterile, green plants were reciprocally crossed with Compana, a two-rowed variety, to test the transmission of gamete abortion and yellow-green seedlings through male and female gametes. The testcross plants, as well as F_2 plants, were grown to maturity in the field.

Pollen mother cells were analyzed by the aceto-carmine smear technique. The proportion of abortive pollen was determined by examining mature pollen in potassium iodide solution. Relative ovule abortion was measured by the proportion of empty florets in the mature spikes.

Experimental Results

Results of selfed progeny tests

The green, partially sterile plants (referred to hereafter as parental type) gave rise to an unusually high proportion of yellow-green seedlings upon selfing. The proportion of yellow-green seedlings observed among 4,471 greenhouse-grown progeny of fifty parental type plants was 40.7 percent. Progeny tests conducted in the field revealed 37.3 percent yellow-green progeny. These yellow-green plants were completely fertile. The slight discrepancy between the field and greenhouse tests was attributed to reduced survival of the yellow-green phenotype under field conditions.

The green plants which constituted approximately 60 percent of the total F_2 from the parental type plants were of three genotypes as revealed in the next generation: (1) Partially sterile plants which produced on selfing a high frequency of yellow-green seedlings (parental type); (2) fertile plants whose progeny derived from self-fertilization segregated 3 green : 1 yellow-green seedling; and (3) fertile plants that gave rise following self-fertilization only to normal green offspring. The frequencies

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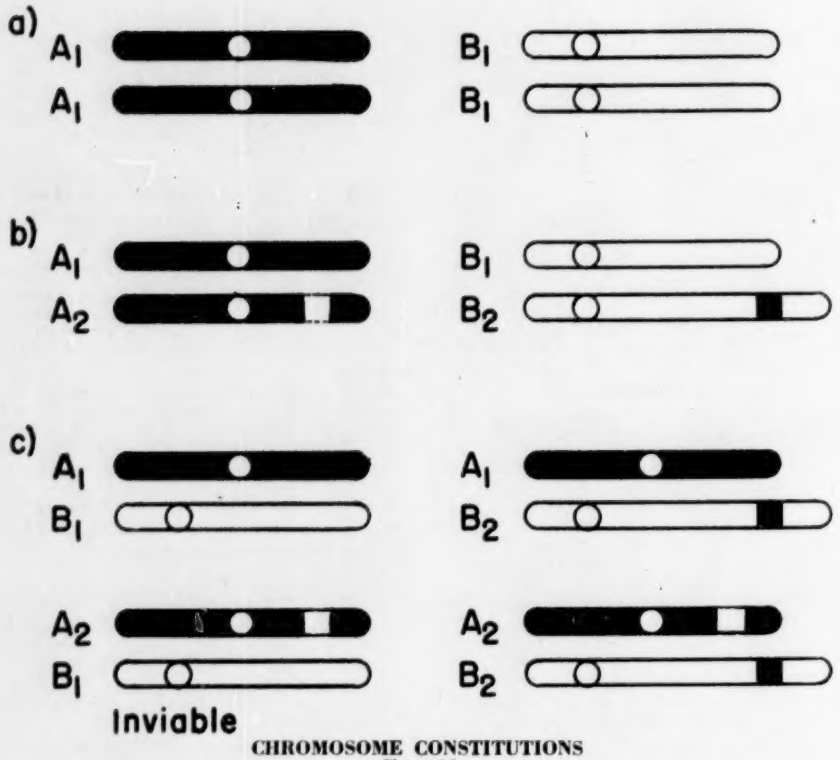


CHROMOSOME CONSTITUTIONS
Figure 16

Postulated chromosome constitution of normal and parental type plants and gametes. The circles represent the centromeres. *a*—Two normal pairs of chromosomes (A_1A_1 and B_1B_1) before irradiation. *b*—Chromosome constitution (A_1A_2 and B_1B_2) of parental type plants showing a non-reciprocal intercalary translocation induced by irradiation. *c*—Four types of gametes involving the normal and translocated chromosomes of parental type plants. A_1B_1, normal; A_2B_1, inviable because of deficiency in A_2; A_1B_2, viable, contains duplicated segment of A_1B_2; and A_2B_2, viable, deficiency in A_2 covered by A_1 segment in B_2.

of these three genotypes are presented in Table I.

Percentages of ovule and pollen abortion in parental type segregants were 29.4 and 26.6, respectively (Table II).

Seven bivalents were observed at the first meiotic division of microsporogenesis in all classes of selfed progenies. No chromosome aberrations were detected upon examination of several hundred cells at metaphase.

Transmission of yellow-green seedling and gamete abortion through male and female gametes

The plants in the progeny derived from the cross to Compana were all green. Examination of their pollen and their progeny derived from self-fertilization revealed the presence of the same three genotypes described above, namely: (1) Partially sterile and producing green and yellow-green seedlings; (2) fertile and producing green and yellow-green seedlings in ratios of 3:1; and (3) fertile and giving rise only to green seedlings. These three types of plants were present in equal frequencies, as shown in Table III. There were no partially sterile plants that produced only green seedlings in their progeny.

Plants of class 1 gave rise to 40.3 percent yellow-green seedlings based on 2,767 out of 6,868 individuals. In addition, they displayed 23.9 percent ovule abortion and 25.9 percent pollen abortion (Table IV). This class was genetically similar to the parental type. Plants of class 2 produced 22.9 percent yellow-green seedlings based on 2,280 out of 9,949 individuals. Plants of class 3 were normal in all measured respects. Thus, the three testcross classes were counterparts of the three green genotypes appearing in the F_2.

Discussion

In a genetic analysis of the foregoing data the following facts must be reconciled:

1. The parental type plants were characterized by the appearance of gamete abortion (25 percent) and a high frequency of occurrence of yellow-green seedlings in their progeny (40 percent).
2. Among the green progeny derived from self-fertilization of the parental type plants, three classes appeared: (1) Parental types, as described above; (2) plants showing no gamete abortion but segregating, upon self-fertilization, green and yellow-green seedlings in a 3:1 ratio; and (3) plants whose genetic behavior was normal in that they showed no gamete abortion and did not segregate yellow-green seedlings.
3. In crosses of parental type plants to plants with normal genic and chromosomal constitutions (variety Compana), the progeny were all green. Plants with the same three types of constitutions appeared as described in 2 above, and they were present in equal frequencies.
4. Mature plants grown from the yellow-green seedlings showed no pollen abortion.
5. In none of the green plants that gave rise only to green seedlings, following self-fertilization, was gamete abortion found.
6. Plants showing gamete abortion always segregated yellow-green seedlings when self-pollinated. No exceptions were found in studies extending through five generations and involving several thousand individuals.
7. In the parental type plants, no obvious chromosomal abnormalities were noted at metaphase of the first meiotic mitosis.

Single or multiple gene mutations and coincidental chromosome changes associated with a point mutation were considered in proposing possible explanations of the foregoing array of facts. However, these possibilities did not conform to the data.

An explanation which appears to account satisfactorily for the above observations introduces the assumption that a small, non-reciprocal translocation occurred between nonhomologous chromosomes. The translocated segment could be inserted at a terminal or intercalary position. In this discussion it has been assumed to be an intercalary insertion, although the position of the transposed segment does not alter the significance of the constructed model. Whatever the nature of the translocation, it had no effect when heterozygous on chromosome pairing at meiosis. Therefore, it is assumed that the segment translocated is small.

The normal chromosomes involved in the non-reciprocal translocation might be designated as A_1 and B_1, the deficient chromosome and the chromosome with the translocated segment as A_2 and B_2, and the chromosome constitution of the parental-type plant as $A_1A_2B_1B_2$ (Figure 16). Only three classes of viable gametes would be expected to be produced by a plant heterozygous for the described transposition (Figure 15). The A_2B_1 carrying gamete is considered to be inviable because of deficiency. This would account for the observed gamete abortion which was approximately 25 percent. The A_2B_2 gametes are functional because the deficiency in A_2 is covered by the translocated segment in B_2. Combinations of the three viable types of male and female gametes produced by self-fertilization result in F_2 genotypes as shown in Figure 17. The observed F_2 genotypes and those expected on this model are given in Table V.

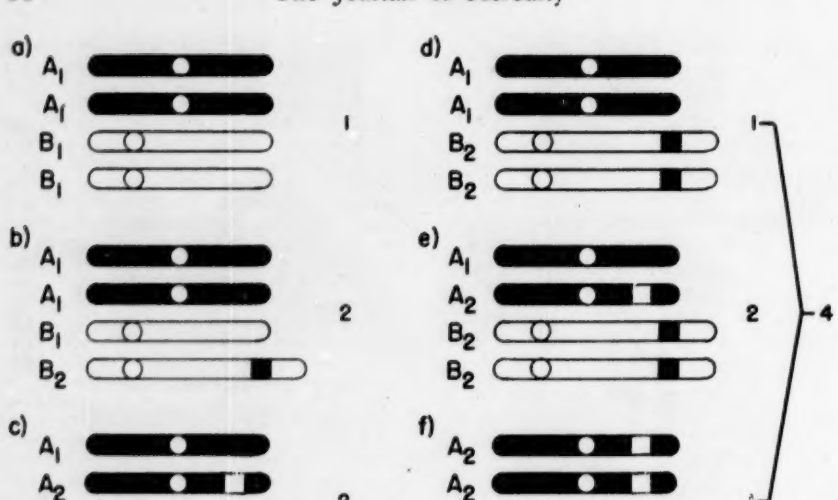
The yellow-green effect was produced when both B_2 chromosomes were present in the nu-

TABLE I. Frequencies of green genotypes arising from self-fertilization of parental type plants. (Greenhouse tests)

Genotype	Frequency Plants	%	% of total F_2
Parental type	52	17.4	22.2
Green, fertile, producing 3 green: 1 yellow-green seedling	55	19.6	23.5
Green, fertile, producing normal plants	32	21.0	13.6

TABLE II. Percentages of ovule and pollen abortion in the four classes of plants derived from self-pollination of parental type plants

Class	Ovule abortion No. plants examined	Ovule abortion Average %	Pollen abortion No. plants examined	Pollen abortion Average %
Yellow-green, fertile	14	4.8	7	2.3
Parental type	38	29.4	11	26.6
Green, fertile, producing 3 green: 1 yellow-green seedling	8	5.9	4	2.6
Green, fertile, producing normal plants	20	4.2	1	2.3



F_2 RATIOS FOLLOWING SELF-FERTILIZATION OF PARENTAL TYPES

Figure 17

Postulated chromosome constitution and ratios of F_2 following self-fertilization of parental type plants: *a*—green, fertile, normal chromosome constitution; *b*—green, fertile, translocated segment in only one chromosome; *c*—green and partially sterile because of A_2B_1 gamete (parental type); *d*, *e* and *f*—yellow-green, fertile, translocated segment present in both B_2 chromosomes, *e*—produces three green: one yellow green in F_2.

cleus. When homozygous, the translocated segment is assumed to inhibit some gene action in the B_2 chromosome. It is possible, however, that a deficiency adjacent to the translocated segment occurred in the B_2 chromosome. This deficiency, when homozygous, may be responsible for the yellow-green phenotype. This latter interpretation, however, requires an assumption of four breaks resulting from the radiation. Furthermore, this deficiency in B_2 must also have had no effects on gamete viability.

Inhibition of gene action in B_2 by the presence of the translocated segment (position effect) would require the assumption of only three breaks since no deficiency in B_2 need be postulated. While this postulate is simpler we cannot, at present, definitely rule out the former possibility.

The three classes of testcross plants derived from reciprocal crosses between parental type plants and plants of the variety Compana would be expected to appear from combinations of the three viable gametes produced by the former plant—A_1B_1, A_1B_2, and A_2B_2, with the A_1B_1 gamete produced by the variety Compana as shown in Figure 18. A comparison between observed and expected genotypes is given in Table VI. The observed values fit

TABLE III. Classes and numbers of testcross plants from reciprocal crosses between parental type plants and Compana

Cross	Partially sterile Producing yellow-green seedlings	Partially sterile Producing only green seedlings	Fertile Producing yellow-green seedlings	Fertile Producing only green seedlings
Parental type plant ♀ × Compana ♂	92	0	95	91
Compana ♀ × Parental type plant ♂	29	0	31	33
Total	121	0	126	124
Percent	32.6	0	34.0	33.4

TABLE IV. Percentages of ovule and pollen abortion in the three classes of testcross plants from reciprocal crosses between parental type plants and Compana

Class	Ovule abortion No. plants examined	Ovule abortion Average %	Pollen abortion No. plants examined	Pollen abortion Average %
Parental type	53	23.9	25	25.9
Green, fertile, producing 3 green: 1 yellow-green seedling	35	4.0	19	2.9
Green, fertile, producing normal plants	36	2.0	24	3.1

the expected very closely on the basis of the model.

The high frequency of appearance of yellow-green seedlings in the progeny of the parental type plants may also be accounted for on the basis of the given model. It may be seen that three different classes of yellow-green plants should be present in the F_2. They were not distinguishable from one another by the methods used in the described study. Investigations of them are now under way.

The given model also can explain the observed relationship between gamete abortion in the parental type plants and the appearance of yellow-green seedlings in the progeny. It shows that the two phenomena are interrelated in origin. Furthermore, the yellow-green phenotype is apparently very stable, since plants of this type have bred true for five generations.

For any type of mutant gene action that one might postulate, there is also a corresponding position effect which might apply as Goldschmidt[1] has emphasized. In the absence of cytological proof it is extremely difficult to rule out one or the other.

The above described situation resembles in some respects that reported by Hagberg, Nybom, and Gustafsson[2] for some of the *erectoides* mutants in barley. Three such mutants among the 28 analyzed (*ert.* 1, *ert.* 7, and *ert.* 14) were inseparable from the translocations they carried. *Ert.* 7, and *ert.* 14 were both associated with breaks occurring at a particular locus in a particular chromosome. Other mutants in barley have been reported by Smith[7] and Moh and Smith[5] to be associated with translocations. This type of association has also been reported in maize by Randolph.[6] St. Lawrence, in private correspondence, has reported a similar situation in *Neurospora crassa* where *albino* 1 is closely associated with a reciprocal translocation.

There is also a very close analogy of the situation described herein with the *pale* locus in *Drosophila melanogaster*[3]. This locus is inseparable from a nonreciprocal translocation and the homozygous duplication is lethal.

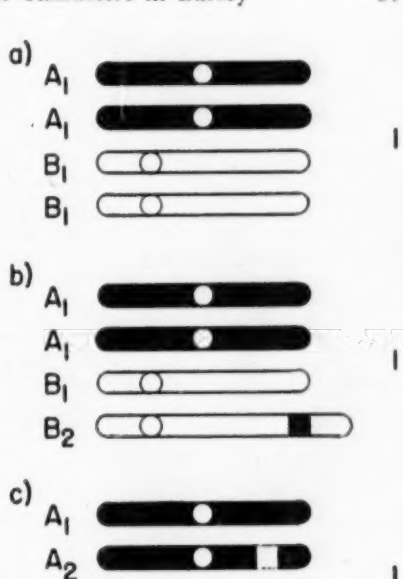
Summary

A mutant line of barley characterized by a high frequency of yellow-green seedlings arising from partially sterile green plants was induced by atomic-bomb radiation at Bikini in 1946. The average percentages of ovule and pollen abortion in the partially sterile green plants (parental type) were 25.8 and 26.6, respectively. Only three types of gametes produced by the parental type plants were viable. Following self-fertilization approximately 40 percent of the progenies from these plants were yellow-green and fertile. The green progenies (approximately 60 percent) of parental type plants consisted of three genotypes: (1) Fertile plants that gave rise following self-fertilization only to normal green offspring; (2) fertile plants whose progeny de-

TABLE V. Chi-square test of F_2 data

	F_2 genotype	Observed %	Expected %
1	$A_1A_1B_1B_1$ green, fertile, producing only green seedling	13.6	11.1
2	$A_1A_1B_1B_2$ green, fertile, producing 3 green: 1 yellow-green seedling	21.5	22.2
2	$A_1A_2B_1B_2$ parental type	22.2	22.2
1	$A_1A_1B_2B_2$ yellow-green, fertile	40.7	44.4
2	$A_1A_2B_2B_2$ yellow-green, fertile		
1	$A_2A_2B_2B_2$ yellow-green, fertile		

$\chi^2 = .924 \quad P = .8\text{-}.9$



TESTCROSS RESULTS

Figure 18

Postulated chromosome constitution and ratios of testcross plants from parental type plants × Compana: *a*—green, fertile; *b*—green, fertile because of translocated segment in only one chromosome; *c*—green, partially sterile because of A_2B_1 gamete (parental type).

TABLE VI. Chi-square test of testcross data

	Testcross genotype	Observed %	Expected %
1	$A_1A_1B_1B_1$ green, fertile, producing only green seedling	33.4	33.3
1	$A_1A_1B_1B_2$ green, fertile, producing 3 green: 1 yellow-green seedling	34.0	33.3
1	$A_1A_2B_1B_2$ parental type	32.6	33.3

$\chi^2 = .066 \quad P = .98\text{-}.95$

rived from self-fertilization segregated 3 green: 1 yellow-green seedling; and (3) parental type.

A small non-reciprocal translocation causing abortion of the deficient gamete class and without apparent effects upon meiotic pairing was postulated to have occurred in the original irradiated material. The yellow-green effect was manifested in plants homozygous for the translocated segment. F_2 and testcross data fit very closely the values expected on this hypothesis. The yellow-green effect was considered to be due to either (1) inhibition of normal gene action in the presence of the homozygous translocation (position effect) or (2) a second deficiency associated with the translocation which did not affect gamete viability.

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TYPE OF HUMAN CANCER, PREVIOUSLY UNOBSERVED IN ANIMALS, FOUND SUITABLE FOR EXPERIMENTAL STUDY

DR. Leroy C. Stevens of the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, has discovered a type of cancer, which, although it occurs in man, until now has been found in no animal suitable for experimental study. Dr. Stevens has found that an inbred strain of mice ("129") develops testicular teratoma (cancer of the testis) in approximately 1 percent of the males born. The discovery holds promise of being an important tool in the study of hitherto unexplored aspects of the biology of these deadly growths. A report of Dr. Stevens' work was recently published in collaboration with Dr. C. C. Little, Director and Founder of the Jackson Laboratory. It is contained in the November issue of *Proceedings of the National Academy of Sciences*, and is exciting considerable interest.

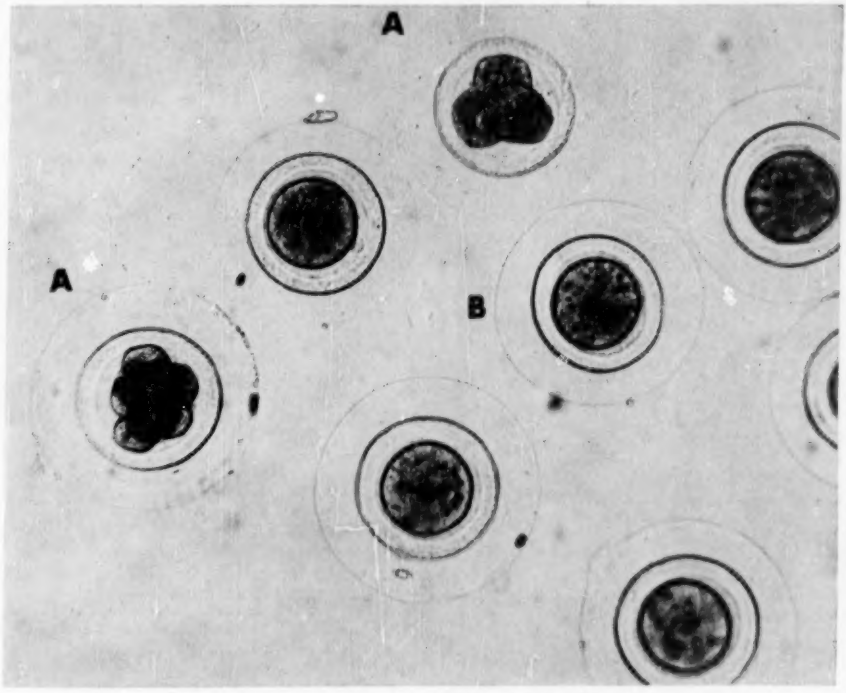
There have been few experimental investigations of teratomas. Descriptions of testicular teratomas other than in man and the horse are rare. Despite several attempts by scientists to gain information leading to a better understanding of these tumors, there is little conclusive evidence pertaining to their origin, the factors causing them, the developmental stage during which they arise, and the actual changes which occur in the tissue during the development of the tumor. The main difficulties in studying large mammals are associated with the rarity and large size of the tumor. Restrictions are also imposed on the study because of the limitations of length of life of the mammal under observation and a long, dormant period between the beginnings of the tumor and its recognition by the scientific observer or physician.

The signicance of discovering a testicular teratoma in a highly useful experimental animal, the inbred mouse, is heightened by the fact that it occurs spontaneously in predictable numbers within its particular strain. It is further enhanced by the fact that the tumor is transplantable and can be continued in successive host animals to prevent its being lost when the host dies.

Included in Dr. Stevens' findings are a number of related facts with important implications for future study, not only of this type of tumor, but of several basic biological problems. Early in the study, it became apparent that the disease in mice probably exists before birth (is congenital). In humans, testicular teratomas have been found in young males which also indicate a congenital origin. They usually do not become clinically detectable until the fourth decade of life. The condition in mice can now be traced to its early stages in the embryo. Likewise, the development of the tumor can be followed progressively throughout its course. Genetic studies may indicate the pattern of its inheritance.

AN EXPERIMENT TO CROSS THE COTTONTAIL AND THE DOMESTIC RABBIT

M. C. Chang and James J. McDonough*

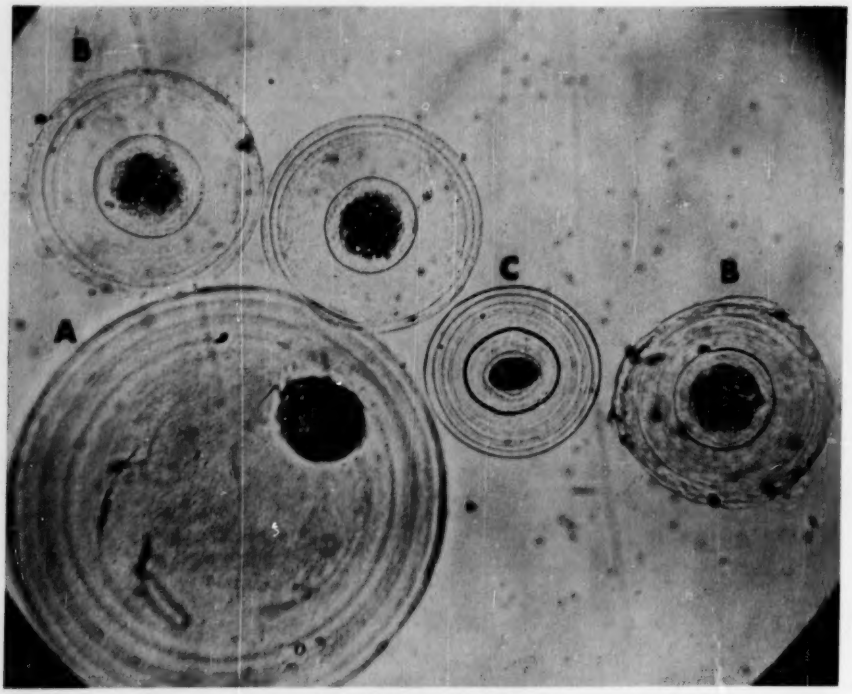


FERTILIZED AND UNFERTILIZED EGGS
Figure 19

Fertilized (*A*) and unfertilized eggs (*B*) recovered from the Fallopian tube two days after artificial insemination of a domestic rabbit with cottontail sperm. ×117.

THE possibility of interspecific hybridization in mammals is a very interesting problem to zoologists in general and to animal breeders and geneticists in particular. In their critical experiments to determine the impossibility of a cross between the hare (*Lepus timidus ainu* and *Lepus europaeus*) and the rabbit (*Oryctolagus cuniculus*), Yamane and Egashira,[6] and Hammond and Walton[4] artificially inseminated the domestic rabbits with sperm of the hare and then waited for delivery. The possibility of fertilization and degeneration was not reported by them. This paper reports an experiment by means of artificial insemination to determine 1) whether the domestic rabbit eggs could

*Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, and Bureau of Wildlife Research and Management, Upton, Massachusetts, respectively. This work was supported by grants from the Rockefeller Foundation and the Josiah Macy, Jr., Foundation.



DEGENERATED EGGS
Figure 20

Degenerated early blastocyst (*A*), degenerated fertilized egg (*B*) and degenerated unfertilized egg (*C*) recovered from uterus seven days after insemination of a domestic rabbit with cottontail rabbit sperm. ×83

be fertilized by cottontail rabbit sperm, 2) whether or not implantation and normal development may occur, and 3) whether reciprocal insemination could be successful. This study was initiated by Dr. C. Thibault of France to determine the possibility of sending cottontail rabbit sperm to France.

Animals and Methods

This experiment was performed in March and May, 1954, during the breeding season of cottontail rabbits. Male cottontail rabbits (*Sylvilagus floridanus*) were trapped in the field and sacrificed in the laboratory just before insemination. The sperm recovered from Vas and epididymis was suspended in Locke's solution. About half a ml. of the suspension, containing about 150 million of very actively motile sperm, was inseminated into the vagina of each domestic rabbit (*Oryctolagus cuniculus*). Intravenous injection of sheep pituitary extracts was given immediately after insemination in order to induce ovulation. Different breeds of domestic rabbits (New Zealand white, Red, Chinchilla, Himalayan, English and Black) kept in the laboratory were used. Two domestic females (Nos. 2 and 3) were inseminated with cottontail sperm suspended in yolk citrate that had been kept at 5-10°C. for one day. Two cottontail rabbits (one *Sylvilagus transitionalis* and one *Sylvilagus floridanus*) were inseminated with the ejaculate of an albino domestic buck and injected intra-

venously with pituitary extracts. These two cottontail rabbits were kept in an outdoor pen for delivery.

Altogether 14 domestic does were inseminated. It was found at operation, however, that four of them had active corpora lutea present in their ovaries. Since fertilization is often inhibited in the presence of active corpus luteum these four animals were discarded; thus only 10 animals were left to determine the possibility of fertilization, blastulation, implantation, normal development and parturition. Since each animal has two uteri, by means of surgical methods, these 10 animals were used as 20. The left Fallopian tube of six animals was removed two days after insemination and flushed with saline. The eggs thus recovered were examined, fixed and stained[1] in order to determine the possibility of fertilization. One uterus of each of six animals was removed seven days after insemination and flushed with saline in order to examine the blastocysts. The uterus of four animals was further examined 16 days after insemination to determine the possibility of implantation and normal development, and finally four animals, after the first operation, were kept for delivery. The number of corpora lutea in the ovary was counted during operation in each animal in order to be sure that they had ovulated. By past experience, it is known that such an operation does not interfere with the development of embryos and implantation.

Results and Discussion

It is quite clear from the results in Table I that when domestic rabbits are inseminated with cottontail rabbit sperm fertilization is possible (Figure 19*A*) but that development fails at the time of segmentation and blastulation before implantation. There were 12 cleaved eggs (37 percent) out of a total of 32 recovered eggs. The cleaved eggs were at the 4 to 12 cell stage (4: 12 cells; 5: 8 cells; 3: 4 cells) with normal nuclei and mitotic figures when examined two days after insemination. Judging from the cleavage rate of rabbit eggs fertilized by rabbit sperm, which should be at about the 32 cell stage two days after insemination, it is evident that the cleavage rate is very slow in these cleaved eggs.

These cleaved eggs were probably fertilized and not parthenogenetically developed, because extra sperm were observed in the perivitelline space of the majority of the cleaved eggs. The number of extra sperm either in the perivitelline space or on the zona pellucida of these fertilized eggs was extremely low, no more than one to four in each egg. In the rabbit eggs fertilized by domestic rabbit sperm, about 30 extra sperm can be located in each egg.[2] It is quite obvious that there is

TABLE I. Cross between domestic rabbit and cottontail rabbit by artificial insemination

Rabbit		No. of ovulation	Fertilization (2 days after insemination) Total	Fert.	Unfert.	Blastulation* (7 days after insemination) Total	Blas.	Fert.	Unfert.	Implantation and development (16 days after insemination)	Parturition
1 Al.	L	5	5	2	3						
	R										no delivery
2 Chin.†	L	8	4	4	0						
	R										no delivery
3 Black†	L	3	1	0	1						
	R										no delivery
4 Al.	L	6	6	3	3						
	R										no delivery
5 Al.	L	7	7	1	6						
	R	6				6		1	5		
6 Red	L	9	9	2	7						
	R	7				5	1	2	2		
7 Him.	L	6				3		1	2		
	R	5								no implantation	
8 Al.	L	3				3		1	2		
	R	4								no implantation	
9 Eng.	L	5				2			2		
	R	6								no implantation	
10 Chin.	L	5				5		2	3		
	R	3								no implantation	
Cottontail											no delivery
Cottontail											no delivery
Total			32	12	20	24	1	7	16		

*All eggs were degenerated.
†Inseminated with cottontail sperm suspended in yolk citrate and kept at 5°-10° for 1 day.

interference in the transportation of cottontail rabbit sperm in the genital tract of female rabbits. This may be partially due to the large size of the female domestic rabbit, which weighs four to six kilos, whereas the body weight of the male cottontail is only about 1 kilo.

In the 20 unfertilized eggs, all of them were uncleaved one-celled eggs (Figure 19*B*). The second maturation spindle had disappeared and the chromosomes were centripetally located in the majority of them. In the 20 unfertilized eggs, three had one sperm in the perivitelline space, one had four sperm in the zona pellucida, eight had one or two sperm in the zona pellucida and eight had no sperm in the zona. It seems that the failure of fertilization may depend upon barriers in the zona pellucida and in the vitelline membrane as well as upon the fact that a small number of sperm ascend to the site of fertilization.

It should be mentioned here that the fertilizing capacity of cottontail sperm was not injured by storage at 5-10°C for one day because fertilized eggs were obtained when two rabbits were inseminated with stored sperm (Nos. 2 and 3).

When six rabbits were examined seven days after insemination, 24 degenerated eggs were recovered from their uteri. There were 16 unfertilized eggs (66 percent) and one degenerated early blastocyst (Figure 20*A*). Judging from the size and appearance of this degenerated blastocyst, it might have been degenerated about 3 or 4 days after insemination, at the time of blastulation. There were seven fertilized but degenerated eggs which were at the 4 to 32 cell stage (Figure 20 *B* and *C*). The very early degeneration of fertilized eggs is probably due to the incompatibility of the nuclear constitution of rabbit egg and cottontail sperm. When four rabbits were laparotomized 16 days after insemination, no embryo development or implantation was observed. The four domestic females inseminated with cottontail sperm and two cottontail females inseminated with domestic rabbit sperm left for parturition showed signs of pseudopregnancy but delivered no young. It indicates that the reciprocal insemination is not successful although the possibility of fertilization is not known.

Attempts to count the number of chromosomes in the testis of cottontail rabbits were unsuccessful. An attempt to obtain two week old embryos for the counting of chromosomes, as performed in the rabbit by Painter,[5] was also unsuccessful because embryonic degeneration at the 13th day was obtained when a cottontail rabbit was artificially inseminated with cottontail rabbit sperm but kept in captivity. However, the number of chromosomes in the germ disk of an implanted cottontail blastocyst recovered six days after artificial insemination was kindly counted by Dr. G. Pincus. The number was over 40 but below 50.

It should be mentioned here that after compiling of the literature on mammalian hybrids, Gray stated "In the few cases examined, no close correlation was found between the chromosome count or the duration of gestation and the ability of species to hybridize."

Summary

Domestic female rabbits were artificially inseminated with cottontail rabbit sperm. About 37 percent of eggs were fertilized but degeneration of fertilized eggs occurred at all of the stages of segmentation before or at blastulation. No young were obtained following insemination of two cottontail females with domestic rabbit sperm.

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